VASOACTIVE EFFECTS OF ERGOT ALKALOID EXPOSURE ON OVINE PEDAL ARTERY AND UMBILICAL BLOOD VESSELS

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ABSTRACT

The incidence of ergot toxicity or ergotism has increased in Western Canada in recent years and resulted in significant economic losses to the livestock farming industry. Chronic ergot toxicity is known to cause severe vasoconstriction through the activation of alpha (α)-adrenergic and serotonergic receptors on vascular smooth muscle cells in end arterioles. Ergotism may also result in abortion. Despite extensive research in the mechanism of ergot toxicity, very little is known about the vascular effects of acute exposure to ergot alkaloids or the mechanism of these effects. Similarly, almost nothing is known about the effects of ergot alkaloids on the umbilical vasculature after 45-days of exposure to ergot alkaloids. Two studies were performed in sheep to address these two important questions in acute and chronic exposure. We hypothesized that acute single-dose exposure to ergot alkaloids increases the contractile response in the pedal artery through the activation of alpha$_1$ (α_1)- adrenergic and serotonergic receptors. We also hypothesized that terazosin (TE), a selective α_1- adrenergic blocker, would abolish the effect of ergot exposure. Our last hypothesis was that a 45-day ergot exposure to ergot alkaloids increases the phenylephrine (PE) contractile response in the isolated umbilical artery and vein as well as the maternal pedal artery.

In the first study, twelve adult sheep were randomly placed into control and exposure groups. The exposure group received a single oral dose of ergot alkaloids obtained from ground sclerotia (600 µg/kg BW) while the control group received only a water placebo. The PE and serotonin contractile responses were assessed in the pedal artery (dorsal

metatarsal III artery) dissected six hours after exposure. The effect of an α_1-adrenergic blocker (TE) was also evaluated. This study demonstrated that acute exposure to ergot alkaloids resulted in a significant increase in PE contractile response compared to the control group (Ctl EC_{50} = 1.74 x 10^{-6} M; Exp EC_{50} = 1.079 x 10^{-6} M, P = 0.046). However, there was no significant difference in serotonergic contractile response (P = 0.12) between the two groups. TE treatment resulted in a significant dose-dependent increase in EC_{50} in both the exposure and the control group ($P < 0.05$ for all treatments). Surprisingly, the effect of TE was significantly more pronounced in the ergot exposed group compared to the control group at two of the three concentrations of TE (TE 30 nM, P = 0.36; TE 100 nM, $P < 0.001$; TE 300 nM, $P < 0.001$). We concluded that the acute effects of ergot exposure are mainly mediated through the activation of α_1-adrenergic receptors and not serotonergic receptors. TE appears to be more potent in blocking the PE contractile response in sheep exposed to ergot compared to the control group. This study may indicate that the dry gangrene seen in sheep, and likely other species, is related to the activation of α_1-adrenergic receptor. This effect may be reversed by using TE especially during the early stages of the disease before cell death occurs. This study may also indicate that the acute single dose exposure scenario may be useful in the study of the vascular effects of ergot alkaloids.

The objectives of the second study were to determine the effects of chronic ergot alkaloid exposure on PE contractile response in the umbilical vasculature, as well as the maternal pedal artery of pregnant sheep. Twelve adult pregnant sheep were utilized in this study, and were randomly placed into groups; six of which received a pelleted diet containing

ergot (46 µg/kg BW) while the control group received the uncontaminated pelleted feed. This study showed that 45-days of oral exposure to ergot alkaloids resulted in a significant increase in PE contractile response in the umbilical artery (Ctl EC_{50} = 3.962 x 10^{-6} M; Exp EC_{50} = 1.161 x 10^{-6} M, $P < 0.0001$) and the umbilical vein (Ctl EC_{50} = 7.889 x 10^{-6} M; Exp EC_{50} = 6.801 x 10^{-7} M, $P < 0.0001$), but not in the maternal pedal artery (Ctl EC_{50} = 4.331 x 10^{-6} M; Exp EC_{50} = 4.856 x 10^{-6} M, $P = 0.3927$). A significantly lower fetal weight was also found in ergot exposed sheep compared to the control group (control, 3.3 ± 0.17 kg; exposure 2.07 ± 0.13 kg, $P = 0.0002$), (T-test, GraphPad Prism). This is the first study to report increased contractility in the umbilical vasculature after 45-days of oral ergot exposure. The mechanism of which is, at least in part, related to the activation of α_1-adrenergic receptors. The concentration selected in this study is below the allowed limits set by the Canadian Food Inspection Agency in sheep which recommends that ergot levels in feed be less than 600 ppb. This study indicates that there is an urgent need to revisit these standards as negative effects can occur at lower concentrations.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

° C	degrees Celsius
5-HT	5-hydroxytryptamine (serotonin)
µM	micromolar
AD	Anno Domini (represents the years after Christ was born)
AIDS	acquired immunodeficiency syndrome
BC	Before Christ (represents the years before Christ was born)
BW	body weight
$CaCl_2$	calcium chloride
cm	centimetres
CA	California
CO	Colorado
CO_2	carbon dioxide
Ctl	control
CWAD	Canada western amber durum
CWRS	Canadian western red spring
EC_{50}	the concentration producing 50% of the maximum response
Exp	exposure
EU	The European Union
g	gram
HPLC/MS	high performance liquid chromatography and mass spectrometry
hrs	hours
KCl	potassium chloride
kg	kilogram
KH_2PO_4	potassium dihydrogen phosphate
LH	luteinizing hormone
LSD	lysergic acid diethylamide
MB	Manitoba

$MgSO_4$	magnesium sulfate
M	molar
mL	milliliter
mm	millimeter
mM	millimolar
MN	Minnesota
NaCl	sodium chloride
$NaHCO_3$	sodium hydrogen carbonate
nM	nanomolar
O_2	oxygen
ON	Ontario
PE	phenylephrine
pH	potential of hydrogen
ppb	parts per billion
ppm	parts per million
SEM	standard error of the mean
SK	Saskatchewan
TE	terazosin
VSMC	vascular smooth muscle cells
US	the United States of America
α	alpha
β	beta

CHAPTER 1: Introduction and Literature Review

1.1 General Introduction

In recent years, there has been an increasing interest in the toxicity related to the consumption of ergot by livestock due to increased incidence and significant economic impact on the farming industry in the US and Western Canada [41, 44]. Ergot toxicity is caused by the chronic ingestion of ergot alkaloids produced by fungal organisms in the family *Clavicipitaceae*. Livestock is exposed to ergot alkaloids through the consumption of grains and grasses infected with *Claviceps* spp or tall fescue infected with *Neotyphodium* spp [6, 37].

The mechanism of ergot toxicity in livestock is thought to be related to the similarity of the structure of ergot alkaloids acting as agonists or partial agonists and sometimes as an antagonist to physiologic monoamine neurotransmitters: epinephrine, serotonin, and dopamine, causing vasoconstriction and inhibition of prolactin secretion [27]. The effects of ergot toxicity can range from subclinical signs such as weight loss and decreased milk production to more severe signs such as necrosis of the tail and loss of the extremities due to dry gangrene [14, 101, 105].

The gangrenous form is the most common clinical manifestation of this disease in livestock and is caused by perturbation of vascular function occurring after chronic exposure. Very little is known about the vascular effects of acute ergot alkaloid exposure

in livestock or their mechanisms. The effects of ergot alkaloids on the umbilical vasculature and the mechanism of these effects are also unknown. This thesis aims to address these two important questions.

1.2 Historical Review of Ergot and Ergotism

The first mention of ergot in history was around 600 BC where it was referred to as "a noxious pustule in the ear of grain" on an Assyrian cuneiform tablet [112]. Another description of ergot appeared in the sacred books of the Parsees around 350 BC where it was described as "a noxious grass that causes pregnant women to drop the womb and die in childbed" [16]**.** The term 'ergot' itself comes from Old French, which means the cock's spur. This term has been used to describe the appearance of the "sclerotium," the ergot-containing fungal body that is black and spur-like in shape [37].

Devastating epidemics of ergotism, also known as St. Anthony's Fire disease, can be traced back to the middle age[7, 105]. During that time, the disease became widespread in many parts of Western Europe and was caused by the consumption of rye bread contaminated with ergot produced by *Claviceps purpurea*. Rye bread, being very cheap, was fed only to the poor during times of famine. To increase the amount of bread produced, and to fulfill the need during the shortage, large amounts of ergot-contaminated grain was used, which greatly exacerbated the epidemic [58, 112].

The consumption of ergot-contaminated feed produces two syndromes: a convulsive and a gangrenous syndrome. The convulsive syndrome was first described in Paris, France, and was associated with the disturbances of the central nervous system causing hallucinations, twitches, and muscle spasms. The gangrenous syndrome was characterized by dry gangrene, necrosis and eventually spontaneous auto-amputation of the appendages. It was first described in Germany [5, 37, 59, 112]. During the epidemics, many died from ergotism, however, some survived. Many of those who survived received treatment at the hospitals of the Anthonines where the malady was believed to be cured by holy water or wine sprinkled with the bone of the Egyptian hermit, St. Anthony (251-356 AD)! It is, however, thought that patients were actually cured because they only ate non-contaminated bread during hospitalization. The gangrenous syndrome was often associated with a burning sensation of the affected appendage and was later called 'Holy Fire,' 'ignis saucer' or St. Anthony's Fire [5, 16, 58].

Ergot also played an important role in human medicine. It was often used by midwives in Europe as a substance to induce parturition, initiate abortion and control postpartum bleeding [16, 37]. This was due to its powerful effect on the uterus producing uterine contraction. However, the use of ergot was discontinued at the end of the 19th century as the complications associated with its use raised many concerns among physicians. Due to the large variation of active ingredients within sclerotia, quite often the accuracy of dosage of the treatment could not be controlled. David Hosack, a physician from New York, described it as 'the pulvis ad mortem', i.e., death powder, as he noticed many

stillbirths associated with its use were related to prolonged uterine contractions during delivery [59].

Since ergot sclerotia had a long history of medical benefits, several attempts were made to reveal the hidden active chemical components. The German pharmacist Heinrich Wiggers was the first to attempt to isolate the active constituents within ergot sclerotia. However, his attempt in 1835, was not successful [4]. Later in 1864, Wenzell described two peculiar alkaloids in rye which he called "Ecboline and Ergotine" and claimed them to be the active ingredients within the ergot sclerotia [4, 58, 59]. In 1875, the first ergot alkaloid was isolated by Tanret ,in Paris, who was able to extract the alkaloid in a crystallized form and named it 'ergotinine' [4].

The discovery of this active ingredient of ergot opened the door for isolating many new active alkaloids. For example, ergotoxine was discovered by Dale and Barger in London in 1906 [59]. However, in 1943, Arthur Stoll and Albert Hofmann demonstrated that ergotoxine was, in fact, a mixture of three alkaloids closely related in chemical structure; ergocornine, ergocristine, and ergocryptine. The same group was later able to isolate ergotamine, which was shown to be a vasoactive [4, 59, 60, 82]. As a medicinal drug, ergotamine was first introduced to human medicine by the work of John Grahm and Harold Wolff who showed that it could be used in patients suffering from migraine headaches in 1938 [5, 59].

Nowadays, the use of ergotamine to treat a migraine has decreased in popularity due to its side effects and the development of more effective and safer drugs. LSD is another ergot alkaloid derivative used in humans after its discovery by Albert Hoffmann in 1983. As a potent hallucinogen, its use was common by secret services who would administer it to people during interrogation [37]. In human medicine, LSD has also been used in the treatment of many neurologic and endocrine disorders because of its action on dopaminergic receptors [34, 60].

Currently, ergotism is rare in human medicine due to the advancement in the cleaning and milling processes, as well as the establishment of regulatory limits for ergot alkaloids in grains. Periodically, patients still suffer from ergot related complications due to the long-term and excessive use of ergotamine for migraine headache treatment and the use of ergot alkaloids in patients with underlying debilitating conditions such as acquired immunodeficiency syndrome (AIDS) or cardiovascular diseases [33]. Nevertheless, ergotism is still seen in veterinary medicine. This devastating disease primarily affects forage grazing animals such as sheep, cattle, and horses [15].

1.3 Ergot Alkaloids: Sources, Lifecycle, Incidence and Allowable limit

1.3.1 Sources of Ergot Alkaloids

Ergot alkaloids are naturally occurring mycotoxins of clinical importance in human and veterinary medicine which are produced by two different genera of fungi in the family of

Clavicipitaceae. This fungal family includes the external spore-producing fungi (*Claviceps* spp.) and endophytic fungi (*Neotyphodium* spp.)[29, 30, 105].

Fungi in the *Claviceps* genus are the main source of ergot alkaloid producing organisms in Western Canada and Europe and will be the focus of our study. The predominant and most prevalent species responsible for economic losses to livestock is *Claviceps purpurea* [11, 64, 68]. This species produces dark, hard, resting, yet infective fungal bodies known as 'sclerotia' which are visible to the naked eye. These fungal bodies contain the pharmacologically active mixture of ergot alkaloids and serve as asexual spores. The most susceptible host plants include barley, wheat, rye and durum, which are commonly grown in Saskatchewan [64]. The clinical syndromes associated with the ingestion of plants infected by *Claviceps* is known as ergotism. In contrast to *Claviceps* spp., the infective stage of *Neotyphodium* spp. are invisible to the naked eye as it develops inside the embryo of the infected seed and remains alive during the lifecycle of the plant. Therefore, microscopic evaluation of suspected plants is required to confirm fungal infection [37].

Claviceps purpurea produces no known benefits to infected plants and is, therefore, considered parasitic in nature. It is thought that the ergot sclerotia may result in the replacement of the seeds causing a significant drop in yield. On the contrary, *Neotyphodium* infection of plants is known to be symbiotic as the infection allows the plant to become more drought, stress, and disease resistant. This infection is most common in the US and is of great economic importance [41, 92]. The clinical syndromes associated with the ingestion of *Neotyphodium* spp. are known as fescue toxicosis. It is

estimated to cost US ranchers more than $860 million annually [41]. Since it is a costly disease to livestock, this syndrome has received great attention in the past 50 years in the US. Although ergotism and fescue toxicosis are clinically similar, the endophyte-infected (*Neotyphodium coenophialum*) tall fescue contains not only a lower alkaloid content, but also a different mixture of alkaloids when compared to the sclerotia of *Claviceps purpurea* [27, 79]. The main ergopeptine alkaloid associated with fescue toxicosis is ergovaline, while the most commonly incriminated alkaloid associated with ergotism is ergotamine [28]. The ergot alkaloid content in samples from rye and wheat flour have shown that the six alkaloids that are abundant in these crops are ergometrine, ergosine, ergotamine, ergocornine, ergocryptine, and ergocristine. Nonetheless, studies conducted to examine the ergot alkaloid content in Canadian rye, wheat, triticale, and barley found that the alkaloid content was different in sclerotia from the same head, field, and region! Ergocristine was the most abundant alkaloid in wheat, barley, and rye from Western Canada while ergotamine was the predominant alkaloid in Eastern Canada [27, 28, 64].

1.3.2 The Lifecycle of Ergot (*Claviceps purpurea*)

The sclerotia are also known as the overwintering external bodies and appear as black cockspur-like seeds of variable sizes which protrude from the plants' florets [30]. Once mature, they become dormant and resistant to harsh environmental conditions allowing them to survive during winter and post-harvest. The size of sclerotia, as well as their alkaloid content, varies significantly depending on the host plants and environmental conditions [56]. The sclerotia fall off to the ground during harvest and can germinate only if

its dormancy is interrupted by several weeks of cold temperature. The preferred environmental temperature for its germination is around 0 °C which should be maintained for at least four to eight weeks, and is ideally followed by a temperature close to 18 °C for several weeks [66]. The germination process can also be interrupted if the soil is too dry, but will proceed as soon as the moist conditions are restored. Germination typically starts in the early spring when the soil is still moist and results in the production of numerous slender bodies known as asci (singular, ascus). Each ascus contains eight ascospores which are typically produced when grasses are flowering [91]. With the help of a wind current, windborne insects and birds, these ascospores reach the stigma of the flowering plant, attach and invade the ovary, and establish a primary infection. In general, plants with a longer duration of flowering are more susceptible to infection. Rye, in particular, is considered very susceptible to infection having not only a long flowering season, but also an open floret structure [64].

Once in the ovary, the ascospores develop into conidia which mix with plant sap arising from the site of infection to form the "honeydew". This sticky syrup attracts flying insects which help spread the infection to other plants. The honeydew containing conidia may also be carried by heavy rain and wind expanding the infected area [37, 64, 66]. The infection established by the honeydew containing conidia is known as the secondary infection. Approximately ten days post-infection, conidia begin to mature and form the rind of sclerotium, which harden and change their shape as time passes becoming the mature sclerotia [37, 66, 91]. See Figure 1.3-1.

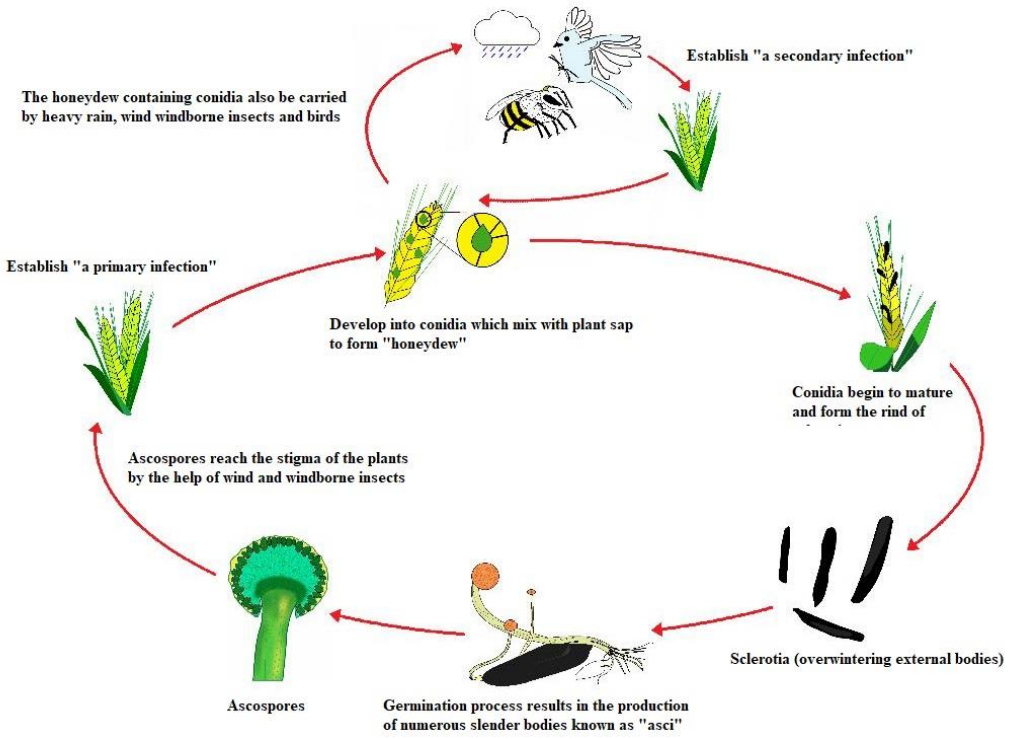

Figure 1.3-1 The lifecycle of ergot (*Clavicep purpurea*) infesting cereals or other grasses. Modified and re-drawn from Menzies et al. (2015)

1.3.3 The incidence of Ergot in Western Canadian Grains

During the 1980s and 1990s, ergotism was not reported as a significant clinical problem for livestock on the Canadian prairies. This was likely due to the absence of sensitive laboratory techniques to detect low concentrations of ergot alkaloid in feed [64]. Therefore, the extent of the problem remains unknown. Canada is known to be the largest exporter of Canadian western amber durum and Canadian western red spring wheat in the world. Crops are grown on over five million acres in Western Canada through Manitoba, Saskatchewan and Alberta [108].

In 1999, the Canadian Grain Research Laboratory (Winnipeg, MB) conducted a survey examining the prevalence of sclerotia in Western Canadian crops [64, 108]. The survey reported that 4% of CWAD and 12% of the CWRS were contaminated by ergot. In Saskatchewan, ergot infection of CWRS was estimated to be 17% with sclerotia representing 0.02% (by weight) of the tested sample. In 2005, a large infestation was reported in Manitoba, where approximately 10% of the CWRS contained ergot sclerotia. Three years later, the ergot infection in the same crop was reported to be significantly higher in Manitoba, Saskatchewan, and Alberta reaching 13%, 15%, and 12%, respectively [64, 108]. The infection rose again in the three provinces in 2011. This time reaching alarming concentrations with 15%, 19% and 29% of the tested samples from these provinces having high amounts of sclerotia. During the same period, ergot contamination of CWAD also rose significantly. In Alberta, the contamination steadily increased from 5% in 2008 reaching 10% in 2012. Ergot infection in Saskatchewan crops

was not steady, but samples taken in 2008 and 2011 yielded 15% and 14% infection, respectively. Data from Manitoba were not sufficient to produce a meaningful pattern of infection due to the small sample size. Overall, the data showed that the incidence and severity of ergot infection increased significantly in both CWAD and CWRS crops during the examination period [64, 108]. A high degree of ergot contamination in crops often results in severe downgrading penalties or even complete rejection of the infected grain, which can be devastating for the industry. Due to the prolonged wet and cold conditions in Western Canada, the number of downgraded samples from crops have increased significantly in recent years [64, 68, 108].

1.3.4 Allowable Ergot Limits in Livestock Feed

There are varied allowable limits of ergot alkaloid content within livestock feed in the literature. The toxicity of ergot alkaloids depends not only on the concentration of alkaloids within the contaminated feed, but also on the specific mixture of alkaloids present as different alkaloids have variable potencies [15, 27, 56]. In cereal grains destined for human consumption, acceptable concentrations of total ergot contamination in the US, Canada, EU and Australia are less than 0.05% by weight [28].

The legislation is in place in Canada which sets the maximum allowable concentrations of ergot alkaloids within cattle and swine feed at 2 to 3 and 4 to 6 ppm, respectively [14]. It is also recommended that pregnant and lactating animals receive a diet with levels less

than 250 ppb to avoid abortion and agalactia. In the US and EU, concentrations that are less than 0.1 and 0.3 ppm total ergot are acceptable [14, 27, 28].

It is worth mentioning that these legislated allowable limits were not established through comprehensive toxicological studies in livestock in most cases. In fact, adverse effects have been reported at levels as low as 100 ppb in livestock depending on overall health status, alkaloid mixture and environmental conditions [27, 28]. The variation in reported adverse effects on livestock health resulted in inconsistent recommendations of tolerable limits. Calves and horses are known to be very susceptible to ergotism whereas poultry have the greatest tolerance[27, 28]. Furthermore, it is important to note that cold weather conditions exacerbate the clinical signs of ergot toxicity [35]. It is, therefore, important to create allowable limits which address the difference in species susceptibility and also take into account climate differences in each region.

1.4 Mechanisms of Action of Ergot Alkaloids

Understanding ergot alkaloids and mechanisms related to their toxicity are challenging considering the variety of factors affecting their biological activity such as the alkaloid concentration and content, types of alkaloid producing plants and the host species. Nonetheless, all ergot alkaloids share a common chemical structure, i.e., the ergoline ring (D-lysergic acid ring structure) which makes their biological activity more predictable [37]. The ergoline ring (Figure 1.4-1a) is the key structure thought to be responsible for toxicity in domestic animals. This is related to the similarity of this ring to active

biological amines in susceptible species including serotonin, epinephrine, and dopamine (Figure 1.4-2).

The mechanism of toxicity and its variability of effects is related to the broad specificity of the interactions between the ergoline ring and the receptors for these active biological amines. These receptors are heterogeneous in nature with at least fourteen subtypes of 5-HT receptors, ten subtypes of adrenergic receptors and five subtypes of dopamine receptors [76]. Thus, ergot alkaloids can act as either agonist, partial agonist or antagonist depending on the types of ergot alkaloids, the location of the receptors in tissue, and the host species.

The ergoline ring is methylated at N-6 and has a functional group on C-8. The substitution of this functional group plays a role in determining the activity of each compound and, likewise, is used to classify ergot alkaloids into three groups: clavines, lysergic acid derivatives, and ergopeptines (Figure 1.4-1b to Figure 1.4-1d). Alkaloids in the group of ergopeptines such as ergotamine and its derivatives are responsible for ergot poisoning and fescue toxicosis in veterinary medicine. The physiological effects seen in animals range from subclinical effects such as decreased weight gain, loss of appetite, changes in thermoregulation and reduced fertility; to pronounced clinical effects such as loss of extremities and abortion [80, 101, 102, 107].

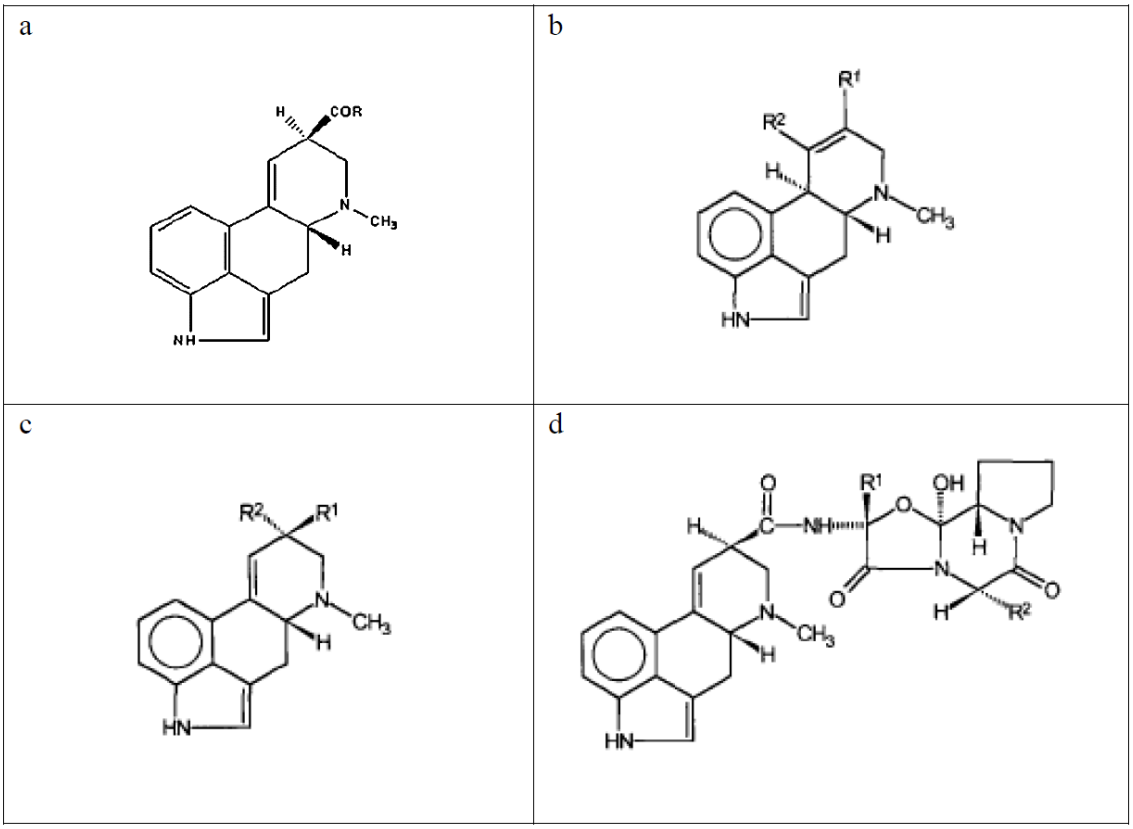

Figure 1.4-1 Chemical structure of ergoline rings as well as three different classes of ergot alkaloids determined by the nature of the functional group at C-8 : (a) Ergoline ring system in ergot alkaloids; (b) represent structure of clavine alkaloids; (c) represent structure of lysergic acid derivertives; (d) represent structure of ergopeptine alkaloids. Modified from Flieger et al. (1997) and Haarmann et al. (2009)

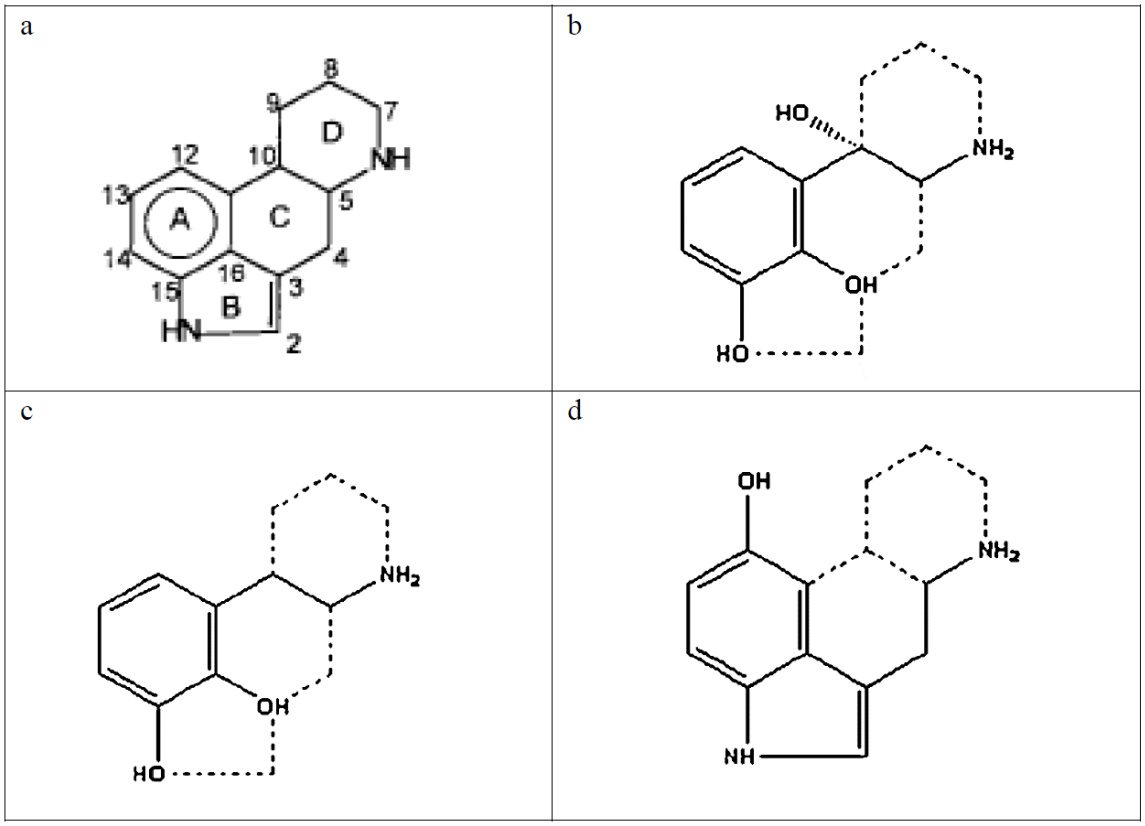

Figure 1.4-2 Comparison of the chemical structures of the ergoline ring system with neurotransmitters : (a) Ergoline ring system in ergot alkaloids (b) chemical structure of noradrenalin (c) chemical structure of dopamine (d) chemical structure of serotonin. Modified from Flieger et al. (1997) and Haarmann et al. (2009)

1.4.1 Effects of Ergot Alkaloids on Serotonergic Receptors

It is known that serotonin, also known as 5-hydroxytryptamine (5-HT), receptors are divided into four main classes: 5-HT_1, 5-HT_2, 5-HT_3 and 5-HT_4. All classes of receptors, except for 5-HT_3, are members of the G-protein-coupled receptors. The 5-HT_3 class belongs to the family of ligand-gated ion channels. These classes of receptors are further divided into different subtypes and, to date, there are fourteen different types of serotonergic receptors each denoted with a letter from A to F after the number [76, 111].

The ergoline ring common to naturally occurring ergot alkaloids allows these alkaloids to bind to the different serotonergic receptors on the vasculature with high affinity, but low specificity, resulting in variable responses at different receptors [76, 111]. Few studies have examined the activation of serotonergic receptors by ergot alkaloids in veterinary medicine. Dyer [21] examined the role of adrenergic and serotonergic receptors in mediating the effects of ergot alkaloids in the umbilical vasculature. Using sheep as a model, he showed that umbilical blood vessels contracted when exposed to serotonin, angiotensin, ergot alkaloids, acetylcholine, epinephrine and norepinephrine and concluded that both receptor types (adrenergic and serotonergic) are present within the umbilical vasculature. Similar to his finding in the umbilical vasculature, he also reported that LSD caused a contraction in the isolated ovine uterine artery at late pregnancy, a response which was abolished by ketanserin, a 5-HT_2 serotonergic receptor antagonist [113]. Using ergovaline, Dyer later showed that 5-HT_2 receptors and not adrenergic receptors produced vascular contraction in isolated bovine uterine and umbilical arteries [22].

In the peripheral vasculature, Oliver et al. 1993 demonstrated that serotonin causes the bovine lateral saphenous vein and the dorsal metatarsal artery to strongly contract [72]. Interestingly, when these isolated arteries were pre-incubated with lysergamide and later exposed to serotonin or adrenergic agonists, the contraction was significantly weakened indicating that lysergamide may act as a partial antagonist at these two locations [72]. Another *in vitro* study also showed that thiabendazole, an approved anthelmintic drug in livestock with antagonistic activity to serotonergic receptors, was able to partially antagonize vasoconstriction induced by serotonin in the bovine pedal vein [73]. Klotz et al. (2012, 2013 and 2016) demonstrated that chronic exposure to endophyte-infected tall fescue caused a significant increase in vascular contractility in the bovine lateral saphenous vein in response to serotonin agonists, a response that was reversible 3 months after the infected diet was withdrawn. He concluded that the main receptor involved in mediating this effect was 5HT-$_{2A}$ [47, 49, 50]. In horses, a single study compared the vascular contractile response of alkaloids within *Acremonium coenphialum* to those of PE and serotonin in the dorsal metatarsal artery and lateral saphenous vein. The study found that the contraction caused by ergot alkaloids was less than that produced by PE and serotonin [45].

In humans, acute migraine attacks are often treated with drugs targeting 5-HT$_{1D}$ [76]. These attacks commonly cause dilatation of extracerebral intracranial arteries resulting in increased vascular pulsation and the stimulation of perivascular branches of the fifth cranial nerve. When 5-HT$_{1D}$ receptors are activated by certain medications, these pathologically dilated arteries contract resulting in symptomatic relief of a migraine

attacks [76]. The use of these drugs is also often associated with increased incidence of myocardial infarction due to their vasoconstrictive effects on coronary arteries [76, 92].

1.4.2 Effects of Ergot Alkaloids on Alpha (α)-adrenergic receptors

The α-adrenergic receptors are a group of G-protein-coupled receptors which are activated by the binding of epinephrine and norepinephrine neurotransmitters. There are two main subtypes of α receptors namely α_1 and α_2. α_1- receptors are further subdivided into α_{1A}, $\alpha_{1B,}$ and α_{1D}. There is no α_{1C} receptor. Similarly, α_2-receptors are divided into four subtypes; α_{2A}, α_{2B}, α_{2C} and α_{2D} [76].

Within the vasculature, the distribution of each subtype is variable. However, in general, α_1-adrenergic receptors are localized postsynaptically within vascular smooth muscle cells and cause vasoconstriction in response to sympathetic stimulation. In contrast, α_2-adrenergic receptors are thought to be located in non-innervated effector cells such as endothelial cells and platelets [85].

Several studies revealed that ergot alkaloids can act on adrenergic receptors. In 1906, Henry Hallett Dale was the first to report that an ergot preparation could cause blood pressure to rise in cats, which is similar to the effect of epinephrine [93]. To his surprise, when these cats were pretreated with ergot and later received epinephrine, blood pressure only fell, which showed that the ergot preparation interacted with epinephrine receptors

and may, in fact, act as blockers! This reversal of epinephrine action by ergot is now known as Dale's vasomotor reversal [59, 85, 93].

Most studies examining the effects of ergot alkaloids on adrenergic receptors focused on α_2-adrenergic receptors. Oliver et al.1998 characterized the adrenergic response to α_1 and α_2 adrenergic agonist drugs in cattle grazing endophyte-infected tall fescue compared to control animals. They concluded that only the α_2-adrenergic receptor is important to mediate the vasoconstrictive effects [74]. Similarly, Roquebert et al. 1986 showed that the vasoconstriction associated with the administration of dihydroergocryptine is mediated by α_2-adrenergic receptors [87]. Others, however, reported that α_1- adrenergic receptor may also play a role to mediate the vasoconstriction. For example, Kalkman et al. 1982 [43] reported that both α_1 and α_2-adrenergic receptors, rather than serotonergic receptors, play a role to mediate vasoconstriction after ergometrine injection in rats. Similarly, Larson et al. 1996 [57] demonstrated that prazosin, which is α_1-adrenergic antagonist, can alleviate syndromes caused by fescue toxicosis in rats.

1.4.3 Effects of Ergot Alkaloids on Dopaminergic Receptors

Ergot alkaloids also act on dopaminergic receptors. Dopaminergic receptors are involved in motor function in the brain and regulate the release of prolactin from the anterior pituitary gland. The repeated activation of D_2 or D_4 dopaminergic receptors within the anterior pituitary gland by ergot alkaloids inhibits prolactin secretion resulting in decreased milk production, delayed or prolonged parturition as well as other disturbances

in the female reproductive system [11, 76]. Luteinizing hormone and prolactin have a synergistic effect in promoting progesterone secretion from the corpus luteum [38]. The disturbance of prolactin secretion could also decrease the progesterone concentration and consequently cause a failure to maintain pregnancy. The depression of serum prolactin can also be used as a bioindicator of ergot alkaloid exposure [81]. In human medicine, ergoline derivatives are also used in endocrine disorders such as hyperprolactinemia secondary to the excessive secretion of prolactin from pituitary prolactinoma [60].

1.5 Clinical Forms of Ergotism in Livestock

Since there is strict regulation of allowable limits of ergot alkaloids in grains used for human food and the advent of improved milling technology, nowadays ergotism is rarely seen in human medicine. Nonetheless, it is still a problem in veterinary medicine, particularly in susceptible species such as beef cattle and small ruminants [27]. It has been reported that the annual economic losses related to this disease in the beef industry in the US were approaching $2 billion in 1993 [41]. A 2013 study found that ergotism in the US beef cattle industry resulted in decreased calf weaning weight by approximately 22.3 kg resulting in losses approaching $500 million every year [44]. In general, ergotism in livestock can be seen in three different forms: gangrenous, convulsive and an “other” form with the later inducing nonspecific clinical signs related to gastrointestinal abnormalities, endocrine dysfunction, poor reproductive performance and abnormal body temperature regulation [6, 11, 14].

1.5.1 Gangrenous Form

Frequently, ergotism in veterinary medicine is seen in the gangrenous form, and this form is frequently observed when ergot concentration exceeds 10 g/kg feed [27]. Early in the course of the disease, animals often show signs of lameness. As the disease progresses in severity, prolonged vasoconstriction leads to dry gangrene resulting in loss of peripheral tissues such as the tail tip, tongue, ear tips and hoof [11, 14]. The speculated pathogenesis of this form of ergotism is caused by the very potent vasoconstrictive properties of the ergot alkaloids such as ergotoxine, which consequently causes vascular injury and circulatory disturbance. One study reported histological lesions in blood vessels after chronic exposure to ergovaline where moderate thickening of the tunica media of tail, skin and lip arterioles was found [71]. It is important to mention that cold weather conditions often result in an exacerbation of the toxic effects with one study reporting dry gangrene after ergot exposure in pregnant sheep kept outdoors in the cold, but not in animals housed indoors [27].

1.5.2 Convulsive Form

The convulsive form of ergotism is mostly caused by the toxins produced by *C. purpurea*, *C. paspali* and the endophyte fungus *Neotyphodium lolii* [14, 110]. This form is often under-diagnosed by veterinarians as animals suffering from this form manifest nonspecific clinical signs such as muscle tremors, incoordination, and paralysis which often mimic other diseases affecting the nervous system [63]. The convulsive form is also

known as ‘ryegrass staggers’ as the disease is associated with the ingestion of endophyte-infected perennial ryegrass [11]. This form is sporadic in Western Canada and EU, but more frequently reported in South Africa and US.

The neurological signs are related to the indole-diterpenoid tremogens, paspalitrem A, B and C, which are commonly produced by *C. paspali* and *N. lolii*. These tremogens can occasionally be detected in the sclerotia of *C. purpurea*, but only in small amounts [110]. Very little is known about the mechanism of action of these tremogens. However, it has been postulated that these toxins inhibit the function of GABA receptors [28].

1.5.3 Other Form

The “other” form of ergotism affects thermoregulation, endocrine function and reproductive performance [6, 11, 14]. During warm weather conditions, blood flow to peripheral organs increases to allow for the dissipation of heat from the body core to the body surface, or skin, through sweating. Chronic exposure to ergot alkaloids often results in widespread peripheral vasoconstriction, thus, limiting the dissipation of heat through the skin resulting in hyperthermia. The animal often responds by hyperventilating, which is clinically seen as rapid and labored breathing, and also by increasing saliva production to dissipate heat [101, 105]. The consequences of prolonged hyperthermia include decreased feed intake, weight loss, reduced fertility, and poor reproductive performance.

The effects of ergot alkaloids on endocrine dysfunction is related to the activation of dopaminergic receptors in the pituitary gland which leads to the inhibition of prolactin secretion[76, 105]. In fact, a persistent decrease in serum prolactin is often used as a biomarker for ergot alkaloid exposure in livestock in general, and mares in particular [81]. The low prolactin concentrations often result in decreased milk production which can be devastating to dairy farmers [11, 76, 105].

1.6 Effects of Ergot Alkaloids on Different Vascular Beds: What We Know from *In Vivo* and *In Vitro* Studies

1.6.1 Peripheral and Visceral Blood Vessels

The most common form of ergot poisoning in livestock is the gangrenous form, which often occurs during the winter and often affects the tail tip and distal extremities [6, 14, 15, 33, 40]. Consequently, peripheral vasculature such as the dorsal metatarsal, lateral saphenous and caudal arteries or veins have been the subject of many classical studies of ergot alkaloid toxicity both *in vivo* and *in vitro*. Other studies focused on visceral arterial vascular beds particularly those related to gastrointestinal absorption.

In vitro studies examining the effects of ergot alkaloids on peripheral vasculature using arterial tissue bath include Klotz et al., Oliver et al. and Abney et al. [45, 47, 49-54, 72, 74, 77]. All of which were discussed previously. Studies examining the clinical effects of ergot exposure on the vasculature include Rhodes et al., 1991 and Aiken et al. (2007 and 2014).

Rhodes et al. 1991 examined blood flow in different tissues using radiolabeled microspheres in sheep and cattle. They showed that castrated sheep receiving a high endophyte diet (0.52 ppm ergovaline) had reduced blood flow to the adrenal gland and the skin covering the inner hindlimb compared to animals receiving low endophyte diet (less than 0.01 ppm ergovaline). In steers, blood flow to the colon, duodenum, cerebellum and the skin covering the ribs was similarly reduced after exposure to a high endophyte diet. Interestingly, steers that previously received a high endophyte diet, but later consumed an endophyte-free diet for 8 days, displayed increased blood flow to the coronary bands of the hoof compared to those consuming a normal diet after a low endophyte diet [84].

Aiken et al. 2009 examined changes in blood flow on cross-sectional areas of caudal artery in heifers after exposure to endophyte-infected tall fescue using Doppler ultrasonography. Compared to baseline, i.e., before exposure, they showed that endophyte-infected tall fescue exposure resulted in decreased caudal artery total cross-sectioned area and blood flow within four hours of exposure. Later, they conducted a study examining the effects of a similar exposure scenario in goats on carotid and auricular arteries. Aiken et al. 2014 again reported decreased cross-sectional area or vasoconstriction at both locations in goats [1, 2].

Reduced blood flow after ergot alkaloid exposure has also been reported in arteries supplying the uterus, ovary and corpus luteum using ultrasound [13, 36]. Other changes in visceral vascular beds, in response to ergot exposure, include the studies from Foote et

al., 2012 and Egert et al., 2014. Using an arterial tissue bath, Foote et al. showed that isolated bovine ruminal artery and vein display vasoconstriction after incubation with ergot alkaloids [32]. In contrast, Egert et al. reported that mesenteric arteries and veins from steers fed endophyte-infected tall fescue for 21-days showed diminished contractility after exposure to some ergot alkaloids, suggesting that the exposure to these alkaloids may alter nutrient absorption [23].

It is interesting to note that the effects of ergot alkaloids on the vasculature are not limited to their role as vasoconstrictors. For example, Strickland et al. 1996 examined the growth promoting effects of ergot alkaloids on VSMC using cell culture. They showed that ergonovine and ergocryptine stimulated VSMC growth while ergovaline inhibited growth [103]. In support, Oliver et al. 2000 showed that chronic exposure to ergot alkaloids resulted in moderate thickening of the tunica media of the arterioles of the tail, skin, and lips histologically [71]. Additionally, Shappell et al. 2003 reported that ergovaline is cytotoxic to Caco-2 cells which mimic gastrointestinal epithelium [96]. Mulac et al. 2011 showed that several ergot alkaloids caused apoptosis when incubated with cultured human kidney cells with ergocristine being the most potent [70].

1.6.2 Umbilical Blood Vessels

Umbilical blood vessels are composed of two umbilical arteries and one umbilical vein. The umbilical vein supplies oxygenated blood and nutrients to the fetal side of the placenta, while umbilical arteries carry out unwanted waste materials and carbon dioxide

from the fetal circulation. It is now well established, from a variety of studies, that umbilical blood vessels lack sympathetic innervation [25, 26, 90]. Therefore, the regulation of vascular tone in the umbilical vasculature is dependent on locally produced and circulating vasoactive catecholamines making them more susceptible to exogenous vasoactive compounds [9].

Quite often, when fetal stress occurs, the level of vasoactive compounds within the umbilical vasculature changes and results in altered flow, which has implications for fetal health [42, 95]. Baker et al. 1992 found that intranasal PE, an inhalant α-adrenergic agonist used in human medicine for respiratory congestion, caused decreased umbilical artery flow velocity. He suggested that this drug should be prohibited in high-risk pregnancies [3].

The effect of ergot alkaloids on the umbilical vasculature has been examined in three studies. In the first study, Dyer demonstrated that serotonergic as well as adrenergic receptors are present in isolated ovine umbilical blood vessels [21]. Later, he showed that ergovaline caused vasoconstriction of ovine umbilical arteries and likely occurs through the activation of 5-HT_2 receptors [22]. A recent abstract by Klotz et al. 2017also showed that ovine umbilical arteries from pregnant sheep fed endophyte-infected tall fescue were very responsive to serotonin but not norepinephrine [55].

CHAPTER 2: Rationale and Hypotheses

2.1 Rationale

Ergot alkaloids are known to cause severe vasoconstriction and dry gangrene of extremities after chronic exposure in livestock. Previous research has indicated that the vasoconstriction is related to the activation of adrenergic and serotonergic receptors by the ergoline ring of ergot alkaloids. Very few studies have examined these vascular effects of chronic exposure in live animals since such long experiments are costly and time-consuming. Very little is known about the acute effects of ergot exposure and whether these effects mimic the chronic exposure scenario. Very few studies have also focused on the feasibility of reversing the vascular effects of ergot alkaloids using pharmacological antagonists. If such antagonists prove useful experimentally, they could be used therapeutically especially in early stages of exposure.

Ergot exposure is also known to cause abortion in pregnant animals or reduced fetal weight in newborns. Since these alkaloids cause vasoconstriction in peripheral vascular bed, it is plausible to think that they have similar effects on the umbilical vein and arteries and may thus alter blood circulation in the fetus.

Two separate experiments were conducted to address the above questions. In the first, to mimic acute exposure, animals were orally exposed to a single oral dose of ergot alkaloids. Since the pedal artery is commonly affected in animals suffering from

gangrene, this artery was selected to examine the roles of α_1- adrenergic receptors and serotonergic receptors in mediating the acute vascular contractile effects. The effect of a selective α_1-adrenergic receptor antagonist was also examined to evaluate its usefulness in reducing the vascular contractile effects of ergot alkaloids.

In the second experiment, pregnant sheep were orally exposed to ergot alkaloids for 45 days. The concentration of ergot alkaloids used in this study mimicked the low dose typically seen in a real exposure scenario. The umbilical artery and vein were dissected after euthanasia and the role of α_1- adrenergic receptors in mediating the contractile effects of ergot alkaloids was examined. The pedal artery of the dam was also dissected and examined similarly.

2.2 Hypotheses

1. Acute single dose oral exposure to ergot alkaloids results in an increased contractile response in the pedal artery and is mediated through the activation of α_1- adrenergic and serotonergic receptors.

2. The acute vascular effects of oral exposure to ergot alkaloids can be reversed via the selective α_1-adrenergic antagonist, TE.

3. Exposure to ergot alkaloids for 45 days results in an increased contractile response in the umbilical artery and vein as well as the maternal pedal artery; an effect that is mediated by the activation of α_1- adrenergic receptors.

2.3 Objectives

1. To examine the acute vascular effects of single-dose oral exposure to ergot alkaloids on pedal arteries and the role of α_1- adrenergic and serotonergic receptors in mediating these effects.

2. To examine the feasibility of reversing the acute vascular contractile effects of single dose oral exposure to ergot alkaloids using a selective α_1-adrenergic antagonist, TE.

3. To examine the vascular contractile effects of 45-day exposure to ergot alkaloids on the umbilical and pedal vasculature and to examine the role of α_1- adrenergic receptors in mediating these effects.

CHAPTER 3: Vasoactive Effect of Acute Ergot Exposure in Sheep

This chapter contains the complete text of a manuscript that will be submitted for publication

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3.1 Abstract

Ergotism is a common and increasing problem in Saskatchewan's livestock. Chronic exposure to low concentrations of ergot alkaloids is known to cause severe arterial vasoconstriction and gangrene through the activation of adrenergic and serotonergic receptors on VSMC. The acute vascular effects of a single oral dose with high-level exposure to ergot alkaloids remain unknown and are examined in this study. This study had two main objectives; the first was to evaluate the role of α_1-adrenergic and serotonergic receptors in mediating the acute vasocontractile response after single-dose exposure in sheep. The second was to examine whether terazosin could abolish the vascular contractile effects of ergot alkaloids.

Twelve adult female sheep were randomly placed into control and exposure groups (n = 6/group). Ergot sclerotia were collected and finely ground. The concentrations of six ergot alkaloids (ergocornine, ergocristine, ergocryptine, ergometrine, ergosine and ergotamine) were determined using HPLC/MS at Prairie Diagnostic Services Inc, (Saskatoon, SK, Canada). Each ewe within the treatment group received a single treatment of ground ergot sclerotia at a dose of 600 µg/kg BW (total ergot) while each ewe in the control group received water. Animals were euthanized 6 hours after the treatment, and the pedal artery (dorsal metatarsal III artery) from the left hindlimb from each animal was carefully dissected and mounted in an isolated tissue bath. The vascular contractile response to PE (α_1-adrenergic agonist) was compared between the two groups

before and after TE (α_1-adrenergic antagonist) treatment. The vascular contractile response to 5-hydroxytryptamine (5-HT also known as serotonin) was also assessed.

Acute exposure to ergot alkaloids resulted in a 38% increase in PE contractile response compared to control (Ctl EC_{50}=1.74 x 10^{-6} M; Exp EC_{50} = 1.079 x 10^{-6} M, P = 0.046). TE treatment resulted in a significant dose-dependent increase in EC_{50} in both exposure and control groups ($P < 0.05$ for all treatments). Surprisingly, TE effect was significantly more pronounced in the ergot exposed group compared to the control group at two of the three concentrations of TE (TE 30 nM, P = 0.36; TE 100 nM, $P < 0.001$; TE 300 nM, $P < 0.001$). No significant difference was found in serotonin contractile response between the two groups ($P > 0.05$).

Similar to chronic exposure, acute exposure to ergot alkaloids results in increased vascular sensitivity to PE, but not serotonin. TE is a more potent dose-dependent antagonist for the PE contractile response in sheep exposed to ergot compared to the control group. This study may indicate that the dry gangrene seen in sheep, and likely other species, might be related to the activation of α_1-adrenergic receptor. This effect may be reversed using TE especially at early stages of the disease before cell death occurs. This study may also indicate that acute single dose exposure scenario may be useful in the study of vascular effects of ergot alkaloids.

Keywords: acute ergot exposure, ergot toxicity, sheep, vasoconstriction, adrenergic receptors

3.2 Introduction

Ergot poisoning remains an economically important disease affecting a variety of animal species including cattle, sheep, horses, and goats with estimated annual losses of more than a billion dollars within the US [41]. Ergot poisoning is caused by the prolonged consumption of ergot alkaloids which are naturally occurring mycotoxins produced by fungi infecting crops such as triticale, cereals, and grains such as barley, wheat and durum [11, 37, 105]. The most widely encountered species of ergot alkaloid producing fungi in Western Canada and Europe are in the family of *Clavicipitaceae* [28, 64, 68]. This fungal family includes the external spore-producing fungi (*Claviceps* spp.) and endophytic fungi (*Neotyphodium* spp.). The major species causing agricultural problems in Western Canada is *Claviceps purpura* [64, 68] . The active ingredients of ergot alkaloids are confined and concentrated within the sclerotia which are external fungal bodies [91]. Clinical signs of lameness, hoof loss, and dry gangrene of the lower limbs, tail, ear tips and teats are commonly seen in chronic ergotism and are related to the effect of ergot alkaloids on the vasculature causing severe vasoconstriction [15, 101, 102].

The precise *in vivo* vasoactive mechanisms of ergot alkaloids have not been determined. However, *in vitro* tissue bath studies, where normal dissected and isolated arterial rings were exposed to purified ergot alkaloids, have previously shown that the adrenergic and serotonergic receptors on vascular smooth muscles are activated. This is also supported by the fact that the chemical structure, i.e., ergoline ring, of ergot alkaloids resembles that

of physiologic neurotransmitters such as dopamine, norepinephrine, epinephrine, and serotonin which are known to be vasoactive [105].

It is important to note that despite the rapid metabolism and excretion of ergot alkaloids which occurs within several hours after exposure [39, 106], the clinical vascular manifestations of ergot alkaloids are always seen after the prolonged (several weeks to months) consumption of ergot-contaminated plants. While these clinical vascular manifestations could be explained by the repeated exposure to ergot alkaloids, these effects often remain long after the ergot-contaminated feed is removed! Recent evidence suggests that ergot alkaloids may bioaccumulate within the vasculature [53, 83]. It is also possible that other unknown vasoactive mechanisms may be involved in mediating these effects.

It is unknown whether acute ergot exposure affects vascular contractility in a similar manner to chronic exposure. If similar effects are found, then acute exposure scenarios may be useful to study the mechanisms of vascular alteration by ergot alkaloids. Many studies have focused on finding an antagonist to counteract the clinical effects of ergotism. For example, dopamine antagonists have been shown to partially reverse the effects of the low prolactin levels produced by ergot alkaloids. Similarly, ketanserin, a 5-HT_2 antagonist, has been reported to reduce the contractile effects of the uterine and umbilical arteries. Elucidating the mechanisms of vascular contractile response induced by ergot alkaloids may prove useful to identify treatment options for the vascular-related clinical manifestations of ergot poisoning.

This study aimed to examine the role of adrenergic and serotonergic receptors in mediating the vascular effects of ergot alkaloids after an *in vivo* acute exposure scenario to these alkaloids. PE (an α_1-adrenergic agonist) contractile response was compared between ergot exposed and control groups before and after TE (α_1 –adrenergic antagonist) treatment. The contractile response of 5-hydroxytryptamine (5-HT, also known as serotonin) was also assessed.

We hypothesized that an acute single dose oral exposure to ergot alkaloids results in an increased contractile response in the pedal artery; an effect that is mediated through the activation of α_1-adrenergic and serotonergic receptors. We also hypothesized that the acute vascular effects of oral exposure to ergot alkaloids can be reversed via TE (the α_1-adrenergic antagonist).

3.3 Materials and Methods

3.3.1 Animals

All protocols were approved by the Animal Care and Ethics Committee at the University of Saskatchewan. Before the experiment, all animals were weighed and clinically examined with body temperature and heart rate recorded. A blood sample was also collected from each animal, and a complete blood count was performed evaluating red and white blood cell counts as well as platelets count and total plasma protein to ensure that all animals were healthy.

3.3.2 Tissue Collection

Twelve healthy adult ewes were randomly assigned into treatment or control groups (*n* = 6 / group). Animals were allowed to acclimatize for fourteen days and were fed alfalfa hay and water *ad libitum*. Ergot alkaloids containing sclerotia were collected, finely ground and the concentrations of six alkaloids (ergocornine, ergocristine, ergocryptine, ergometrine, ergosine and ergotamine) were determined using HPLC/MS at Prairie Diagnostic Services Inc., (Saskatoon, SK, Canada) [56]. Each ewe within the treatment group received a single dose of ground ergot sclerotia at a dose of 600 µg/kg BW (total ergot) dissolved in 50 mL of water via a stomach tube. The concentrations of ergot alkaloids within sclerotia are recorded in Table 3.4-1. The control group received water placebo. Six hours after treatment animals were euthanized using a captive bolt, and a necropsy was performed. A 15 cm segment of the pedal artery (dorsal metatarsal artery III) was carefully dissected and collected from each animal, soaked in a diluted heparin solution (10 Unit/1mL) and transferred into a container containing modified Krebs-Henseleit buffer solution [in mM: 118 NaCl, 4.7 KCl, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 22.0 $NaHCO_3$, 5.0 glucose and 2.5$CaCl_2$; Sigma-Aldrich Canada Ltd. (Oakville, ON, Canada) (pH 7.4 gassed with 95%O_2, 5%CO_2 at 37˚C)] on ice until transport to the laboratory. Immediately upon arrival to the lab, adipose and connective tissue were carefully removed from each arterial segment and sliced into four 3 to 5 mm cross sections. Each arterial section was suspended between the bases of two triangular-shaped wires within an isolated 10 mL tissue bath (Chengdu equipment manufacturing, China) containing modified Krebs-Henseleit buffer solution maintained at the above conditions. Arterial

rings were allowed to equilibrate for 1 hour under a resting tension of 2 grams with the bath solution changed every 15 minutes. Arterial rings were treated with PE (1×10^{-6} M) (Sigma-Aldrich Canada Ltd. Oakville, ON, Canada) to initiate contraction and to confirm tissue viability and responsiveness. The tissues were later washed with incubation buffer until resting tension was achieved.

3.3.3 Contractile Response

Three vascular rings from the pedal artery of each animal were used to assess the PE contractile response before and after the incubation of each ring with a different concentration of TE. The vascular contractile response to 5-HT was also assessed in the fourth arterial ring. Initially, a cumulative concentration-dependent contraction in response to PE was obtained by adding increasing concentrations of PE (1×10^{-9} M to 1×10^{-4} M). After each PE treatment, arterial rings were allowed to achieve maximum tension which plateaued for 2 minutes before the next concentration was added. After the last PE treatment, arterial rings were allowed to return to resting tension with buffer replacement occurring every 15 minutes for 1 hour. This was followed by incubating each of the three rings with 30, 100 or 300 nM TE for 20 minutes after which the cumulative PE contractile response was repeated in each chamber as above. A similar cumulative contractile response for 5-HT was obtained in the fourth ring by adding increasing concentrations of 5-HT (1×10^{-9} M to 1×10^{-4} M). Following completion of the exposure, all rings were exposed to 1×10^{-6} M PE to verify their viability.

3.3.4 Data Collection, Analysis and Statistical Analysis

All measured isometric contractile responses were recorded in grams of tension using 'Chart' software and Powerlab equipment (AD Instruments Inc., Colorado Springs, CO, USA). For each PE or 5-HT treatment, the maximum tension in grams achieved before the 2 minutes plateau period was recorded and corrected for a baseline. To minimize variation due to arterial size, each contractile response from an individual ring was normalized to its maximum contractile tension induced by 1×10^{-4} M PE or 5-HT treatment.

Contractile response data were presented as percentage means ± *SEM* of the maximum contractile effect induced by 1×10^{-4} M PE or 5-HT treatment. For each treatment type, a sigmoidal dose-response curve was plotted using nonlinear regression with variable slope utilizing GraphPad Prism 7 (GraphPad Software Inc., La Jolla, CA) which was later used to calculate potency presented as the concentration producing 50% of the maximum response (EC_{50}). Results were presented as the log of the EC_{50} value. Statistical differences in EC_{50} among the different dose-response curves were calculated by the extra sum-of-squares F-test were a *P*-value less than 0.05 was considered significant.

3.4 Results

All animals remained healthy after treatment and did not exhibit any clinical signs during the 6 hours period between the administration of ergot alkaloids and euthanasia. No gross or histological changes were seen in either group in the lung, liver, kidneys, heart, spleen, intestines, fat and pedal arteries.

3.4.1 Phenylephrine Dose Response Curve Compared Between Ergot Exposure and Control Groups

In the control group, the PE contractile response was first observed at 1×10^{-7} M concentration, and the maximum contractile response recorded at the highest PE concentration (1×10^{-4} M*)* was 22.8 grams. The contractile response in the exposure group was first observed at 0.5×10^{-7} M while the highest PE concentration yielded a maximum contraction of 18.0 grams. Ergot exposure resulted in a significant decrease in EC_{50} compared to the control group ($P = 0.0462$). Comparisons of PE contractile responses between the two groups are presented in Figure 3.4-1. Details of EC_{50} are for all groups are presented in Table 3.4-2.

3.4.2 Effect of Terazosin Treatment on Phenylephrine Dose Response Curve

In the control group, TE treatment resulted in a significant and dose-dependent increase in EC_{50} ($P < 0.0001$ for all concentration; 30, 100 and 100 nM). Similarly, EC_{50} significantly increased in a dose-dependent manner in the exposure group after terazosin

treatment ($P < 0.0001$ for all concentration; 30, 100 and 100 nM). See Figures 3.4-2 and 3.4-3 for details. The blocking effect of TE was greater in the exposure group when compared to the control group when given at 100 nM and 300 nM ($P < 0.0001$). See Figures 3.4-5 and 3.4-6. A similar trend of increasing EC_{50} in the exposure group compared to the control group after the 30 nM TE treatment was seen, but the difference was not statistically significant ($P = 0.076$), Figure 3.4-4. (See Table 3.4-2 for details.)

3.4.3 Serotonin Dose Response Curve Compared Between Exposure and Control

No significant difference was found in EC_{50} compared between the control and exposure group.

3.4.4 Results Tables

Table 3.4-1 The concentration of six ergot alkaloids determined within ground sclerotia using HPLC/MS*. The total concentration of these alkaloids was used to formulate a single oral dose (600 µg/kg BW) which was administered to each sheep using a stomach tube.

Alkaloid	Concentration (ppb) Dry weight	Oral dose (µg/kg BW)
Ergocornine	216500	26.4
Ergocristine	3653000	445.9
Ergocryptine	540100	65.9
Ergometrine	78850	9.6
Ergosine	89570	10.9
Ergotamine	338300	41.3
Total	4915900	600

*The detection limit for each alkaloid was 1.25 ppb.
HPLC/MS, high performance liquid chromatography and mass spectrometry; µg/kg BW, microgram per kilogram body weight; ppb, part per billion

Table 3.4-2 PE EC_{50} compared between ergot exposed and control sheep (n = 6/group) before and after terazosin treatment in dissected pedal arteries using an arterial tissue bath. Ergot exposed sheep received a single oral dose of 600 µg/kg BW total ergot dissolved in a water based on the levels of six ergot alkaloids determined previously. Control sheep received a water placebo treatment. The effect of terazosin was determined using three increasing concentrations of terazosin; 30,100 and 300 nM. The serotonin EC_{50} was also determined in dissected pedal arteries and compared between the two groups. For each treatment type, a sigmoidal dose-response curve was plotted using nonlinear regression which was used to calculate EC_{50}. Statistical differences in EC_{50} among the different treatment types were calculated by the extra sum-of-squares F-test. A *P*-value less than 0.05 was considered significant.

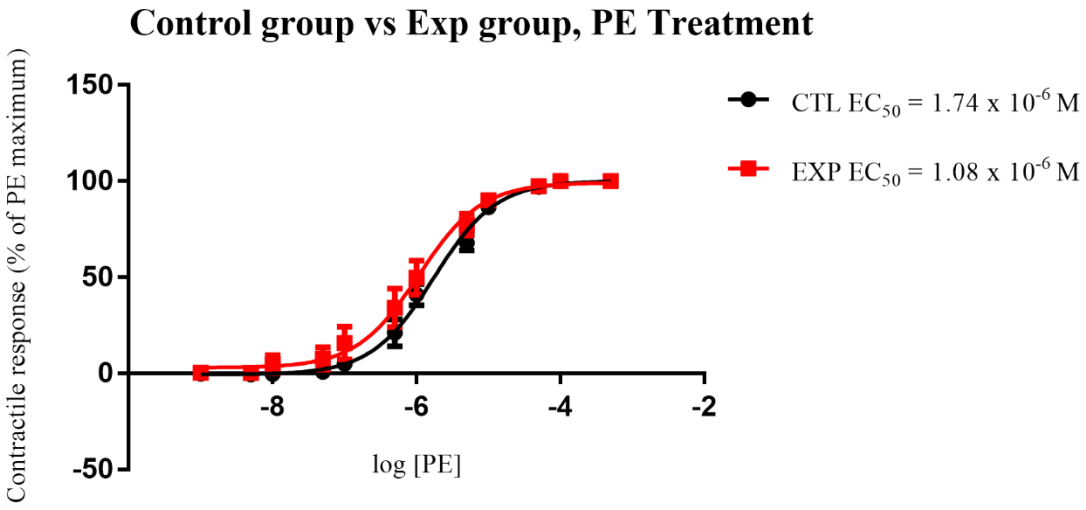
Tissue bath Treatment Type	**Control EC_{50} and 95% CI**	**Ergot Exposed EC_{50} and 95% CI**	***P* -value**
PE	1.74×10^{-6} (1.39×10^{-6} - 2.18×10^{-6}) [a, d]	1.08×10^{-6} (7.4×10^{-7} - 1.57×10^{-6}) [a, g]	$P < 0.05$
PE + TE (30nM)	6.11×10^{-6} (4.78×10^{-6} - 7.8×10^{-6}) [d, e]	7.74×10^{-6} (4.63×10^{-6} - 1.3×10^{-5}) [g, h]	$P = 0.37$
PE + TE (100nM)	9.6×10^{-6} (7.69×10^{-6} - 1.2×10^{-5}) [b, e, f]	2.57×10^{-5} (1.7×10^{-5} – 3.9×10^{-5}) [b, h, i]	$P < 0.0001$
PE + TE (300nM)	1.77×10^{-5} (1.34×10^{-5} - 2.33×10^{-5}) [c, f]	6.73×10^{-5} (4.24×10^{-5} – 1.07×10^{-4}) [c, i]	$P < 0.0001$
5-HT	5.01×10^{-7} (2.3×10^{-7} – 1.09×10^{-6})	1.65×10^{-6} (7.19×10^{-7} – 3.77×10^{-6})	$P = 0.12$

[a, b, c, d, e, f, g, h, i] letters with the same superscript are significantly different

EC_{50}, the concentration of phenylephrine producing 50% of the maximum contractile response; µg/kg BW, microgram per kilogram body weight; PE, phenylephrine; TE, Terazosin; CI, confidence interval; 5HT, 5-hydroxytryptamine (serotonin); nM, nanomolar

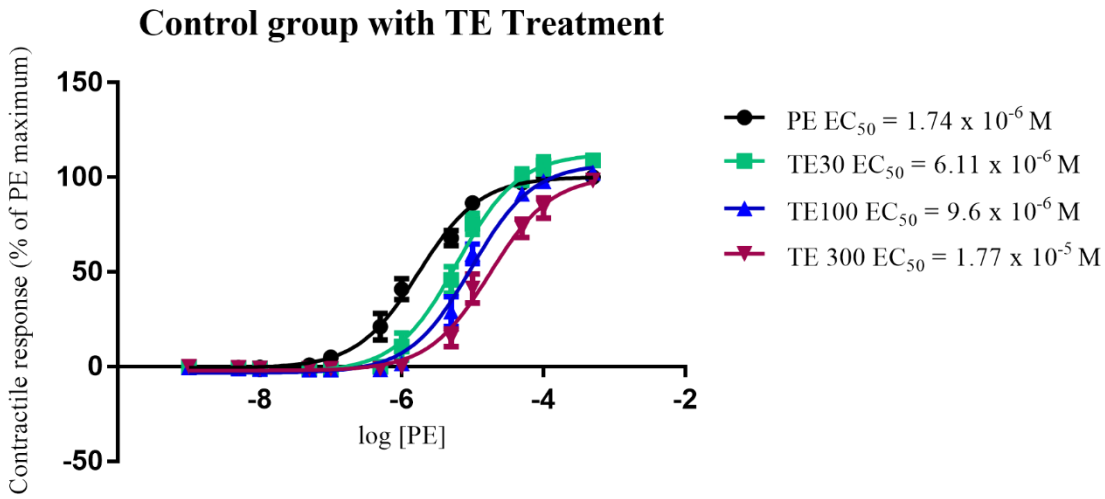
3.4.5 Result Figures

Figure 3.4-1 Mean arterial contractile responses to increasing concentration of PE compared between control and ergot exposed group. The pedal artery was collected 6 hours after single oral exposure to 600 µg/kg BW (total ergot) or after placebo water treatment (n = 6 / group). Contractile response data were presented as percentage means ± *SEM* of the maximum contractile effect induced by 1 x 10^{-4} M PE treatment. Ergot exposure resulted in a significant decrease in EC_{50} compared to the control group (P < 0.05).



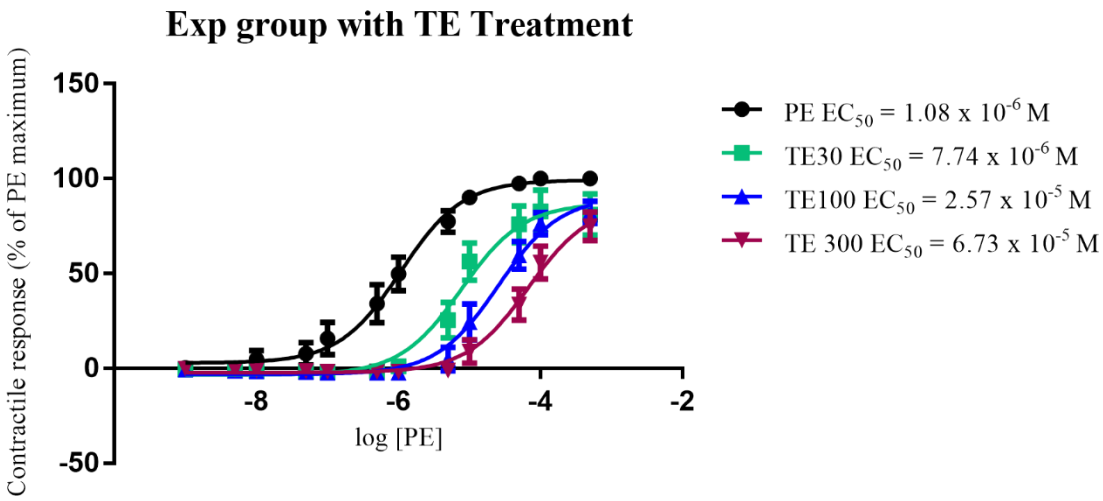
EC_{50}, the concentration of phenylephrine producing 50% of the maximum contractile response; PE, phenylephrine; BW, body weight; CTL, control group; EXP, exposure group; M, Molar

Figure 3.4-2 Mean arterial contractile responses to increasing concentration of PE in control animals compared before and after 30, 100 or 300 nM TE treatment (n = 6). Contractile response data were presented as percentage means ± *SEM* of the maximum contractile effect induced by 1 x 10^{-4} M PE treatment. TE treatment resulted in a significant dose-dependent increase in EC_{50} compared to PE alone ($P < 0.0001$).



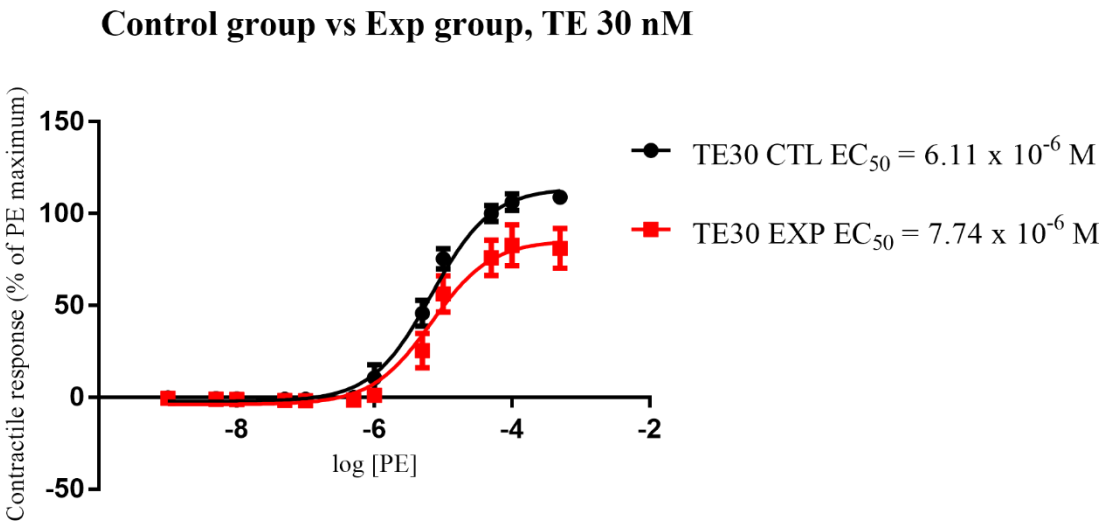
EC_{50}, the concentration of phenylephrine producing 50% of the maximum contractile response; PE, phenylephrine; TE, terazosin; M, Molar; nM, nanomolar

Figure 3.4-3 Mean arterial contractile responses to increasing concentration of PE in ergot exposed animals compared before and after 30, 100 or 300 nM TE treatment (*n* = 6). Contractile response data were presented as percentage means ± *SEM* of the maximum contractile effect induced by 1 x 10^{-4} M PE treatment. TE treatment resulted in a significant dose-dependent increase in EC_{50} compared to PE alone ($P < 0.0001$).



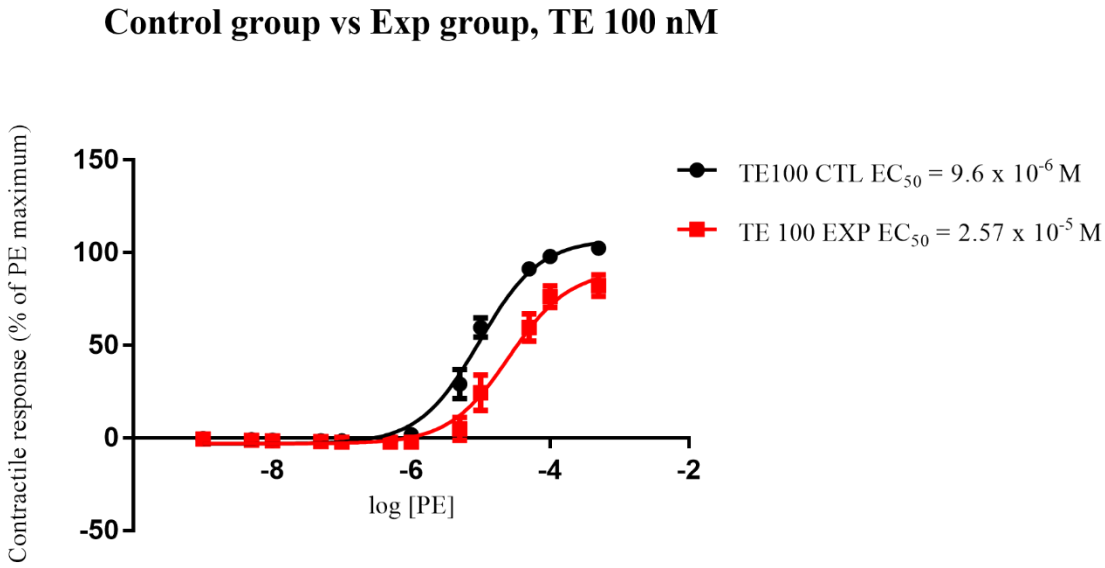
EC_{50}, the concentration of phenylephrine producing 50% of the maximum contractile response; PE, phenylephrine; TE, terazosin; Exp, exposure group; M, Molar; nM, nanomolar

Figure 3.4-4 Mean arterial contractile responses to increasing concentration of PE compared between control and ergot exposed groups after TE treatment at 30 nM. EC_{50} was not significantly different between the two groups (P = 0.37). Contractile response data were presented as percentage means ± *SEM* of the maximum contractile effect induced by 1 x 10^{-4} M PE treatment.



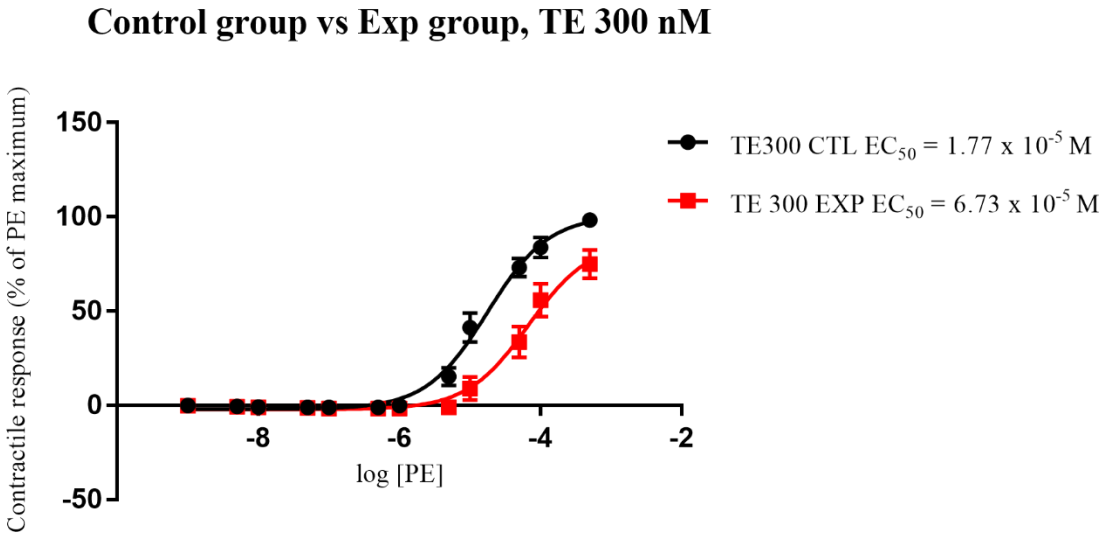
EC_{50}, the concentration of phenylephrine producing 50% of the maximum contractile response; PE, phenylephrine; TE, terazosin; CTL, control group; EXP, exposure group; M, Molar; nM, nanomolar

Figure 3.4-5 Mean arterial contractile responses to increasing concentration of PE compared between control and exposure after TE treatment at 100 nM. EC_{50} was significantly different between the two groups ($P < 0.0001$). Contractile response data were presented as percentage means ± *SEM* of the maximum contractile effect induced by 1 x 10^{-4} M PE treatment.



EC_{50}, the concentration of phenylephrine producing 50% of the maximum contractile response; PE, phenylephrine; TE, terazosin; CTL, control group; EXP, exposure group; M, Molar; nM, nanomolar

Figure 3.4-6 Mean arterial contractile responses to increasing concentration of PE compared between control and exposure after TE treatment at 300 nM. EC_{50} was significantly different between the two groups ($P < 0.0001$). Contractile response data were presented as percentage means ± *SEM* of the maximum contractile effect induced by 1 x 10^{-4} M PE treatment.



EC_{50}, the concentration of phenylephrine producing 50% of the maximum contractile response; PE, phenylephrine; TE, terazosin; CTL, control group; EXP, exposure group; M, Molar; nM, nanomolar

3.5 Discussion

The biological effects of ergot alkaloids on livestock are known to be diverse. This diversity is not only related to differences in alkaloid concentration and specific alkaloid in different plants, but also due to their ability to affect multiple biological processes[27, 28, 105]. The ergoline ring system, which is a structure common to ergot alkaloids, is similar to the ring structure of epinephrine, dopamine, and serotonin thus allowing ergot alkaloids to mimic their function. In the vasculature, ergot alkaloids bind with a variety of serotonergic and adrenergic receptors to modify vascular tone [15]. The effects of these alkaloids are known to be diverse with differing potencies among different animal species, however, within a species, the effects are dependent on the animal's general health, body condition, reproductive status and previous exposure [104, 105].

Ergotism in livestock is known to cause dry gangrene due to severe vasoconstriction within peripheral vasculature. Ergotism occurs after the prolonged ingestion of ergot alkaloids. Therefore, previous studies have focused on examining the mechanisms of vasoconstriction following chronic exposure scenarios [23, 31, 47]. Thus, the vascular effects following acute exposure remain unknown. In this study, we wanted to investigate the role of α_1-adrenergic receptor activation on vascular contractile response following a single acute high-dose of oral exposure scenario to ergot alkaloids using sheep as a model. Similar to other livestock species, sheep are chronically affected by ergotism and develop dry gangrene after prolonged exposure. We chose to examine the pedal artery due to its peripheral location on the ovine limb.

Ideally, pure individual ergot alkaloids should be used in prolonged feeding trials to precisely examine their vascular effects and the mechanism of these effects. However, because pure ergot alkaloids are very expensive, previous studies often used ergot or endophyte-infected tall fescue. It is often difficult to accurately estimate the individual dose in these studies as the concentration of alkaloids within feed is subject to significant variability due to feed storage conditions and uneven distribution. Alternatively, pure individual alkaloids are often used on dissected arteries to examine their vascular contractile effects *in vitro* using arterial tissue bath systems. In order to achieve a more defined dosing protocol, we used ground sclerotia in which the concentration of six different ergot alkaloids was determined [27, 56]. The dose was adjusted in every animal depending on the body weight to receive a dose of 600 µg/kg BW of total ergot alkaloid content.

It is known that the degree of vasoconstriction induced by ergot alkaloids is alkaloid dependent. For example, the vasoconstrictive effect elicited by ergocryptine is 100 times less potent as compared to ergotamine whereas ergocristine and ergocornine are 10 times less potent. Ergovaline, the predominant alkaloid in tall fescue grass, is thought to have a similar potency to ergotamine [15, 94]. The potency of these alkaloids varies depending on their relative binding affinity to α-adrenergic and serotonergic receptors and their ability to specifically activate them. Most studies examining the vascular effects of ergot alkaloids have focused on studying the serotonergic receptors [22, 47, 50, 72]. However, very few studies examined the activation of α-adrenergic receptors by different alkaloids. For example, the contractile response in the lateral saphenous vein of cattle grazing tall

fescue was significantly enhanced compared to control animals by BHT-920, an α_2-adrenergic agonist, but not by PE (α_1-adrenergic agonist) [74]. In addition, Schöning et al. reported that ergovaline stimulated α_1-adrenergic receptors but with low efficacy in rat thoracic aorta [94]. *In vivo* studies focusing on heart rate and blood pressure changes after exposure to ergot alkaloids also indicate α-adrenergic receptor activation. Bradycardia induced by ergotamine in anesthetized rats was reduced by yohimbine, an α_2-adrenergic antagonist. In addition, ergotamine treatment reduced the tachycardia induced by electrical stimulation of the spinal cord, and the reduction was similarly blocked after yohimbine treatment [88]. Similarly, in rats, ergotamine has been shown to act as an agonist on α_2-adrenergic receptors and an antagonist on α_1-adrenergic receptor [87]. In our study, a significant increase in PE contractile response was found in ergot exposed sheep compared to control animals which might suggest that α_1-adrenergic receptors mediate that response.

Similar to what we expected, TE decreased the PE contractile response in ergot exposed and control sheep due to its antagonistic effects on the α_1-adrenergic receptor. However, surprisingly, the potency of TE as an α_1-adrenergic receptor antagonist was significantly enhanced in ergot exposed sheep compared to controls. It has been recently shown that previous exposure to high concentrations of ergot alkaloids may decrease vascular contractility making the vasculature less susceptible to the effects of ergot alkaloids. Klotz et al. examined the contractile response to ergovaline in cattle chronically grazing high and low-endophyte-infected tall fescue [50]. This study demonstrated that the maximum contractile response was significantly higher in steers consuming low-

endophyte-infected tall fescue. This is contrary to other studies, which found that the increase in vascular contractile response to ergot alkaloids is dose-dependent [61, 72]. It is, thus, possible that the vascular contractile effect of ergot alkaloids is dose-dependent but may become less effective at very high doses. It is possible that the high dose of ergot alkaloids we used resulted in a relatively low contractile response, and also enhanced the blocking effect of TE resulting in a reduced contractile response compared to control tissues.

Alternatively, it is also possible that the effect of the blocker was enhanced in the ergot exposed group due to the unique mixture of alkaloids in the diet. Interestingly, it has been shown that the presence of ergocristine, ergocornine and ergocryptine together produces adrenergic blockade [24, 100, 109]. Additionally, Roquebert and Demichel reported that ergocristine acts as an α_1-adrenergic blocker in rat tail artery [86, 87, 89]. Ergocristine had the highest concentration in the diet used in this study and may have acted as an antagonist. The enhanced blocking effect of TE in ergot exposed animals may indicate that this blocker may be useful in counteracting the vascular effects of ergot alkaloid exposure.

Several studies have shown that ergot alkaloids interact with serotonin receptors in chronic exposure scenario [22, 47, 72]. Contrary to this, we found no significant difference in contractile response between the two groups suggesting that serotonin may not affect vascular contractility after acute exposure. In agreement with our finding, Kalkman et al. reported that in rats injected intravenously with ergometrine the vasoconstrictor response was related to the activation of α_1- and α_2-adrenergic receptors, but not serotonergic

receptors [43]. High-level exposure in ruminants can result in nervous signs such as hyperexcitability, hypermetria and tremors [15, 17]. The dose we used was relatively high, but was well tolerated by all animals with none showing clinical signs of illness.

Currently, ergot toxicity is thought to be only related to the prolonged consumption of ergot alkaloids, and it is presumed that a short-term exposure will have no significant clinical effects. However, we show for the first time that even a single oral dose of ergot alkaloids causes a significant contractile response in arteries supplying distal extremities. This finding is of significance to the livestock industry and regulators as it may indicate that in cold weather conditions, short-term exposure to ergot alkaloids may result in significant decrease in blood supply to the extremities making animals prone to gangrene. It would be interesting to examine whether a similar but lower level of exposure scenario would result in a similar contractile response in livestock. In addition, it is also important to examine the effects of a short-term exposure on other systems as it is now presumed that the effects are only seen after chronic exposure. If similar negative effects are seen in other systems, it may indicate the need to lower the allowable limits of ergot alkaloids within feed to reflect the true nature of the negative impact of this disease.

The finding that a blocking effect of TE was more potent in ergot exposed animals may indicate that this drug could be used to treat animals who have been recently exposed to ergot alkaloids. If it is proved to be useful, this drug may significantly reduce the economic impact of ergotism to the livestock industry. It also would be interesting to examine whether TE has any impact on other systems affected by this disease.

In summary, this study found that acute high-level exposure to ergot alkaloids results in increased vascular sensitivity to PE, but not serotonin and increases the effect of TE. Additional studies are immensely needed to examine the role of adrenergic, serotonergic and other receptors both in *in vivo* and *in vitro*.

3.6 Acknowledgement

This study could not have been possible without the collaborative efforts from Dr. Desai and Dr. Jadhav for their expertise and guidance in tissue bath experiment, as well as Jair Gobbett for his dedicated work during the clinical trial.

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PREFACE TO CHAPTER 4

In the previous chapter, we demonstrated that acute ergot alkaloid exposure resulted in significant increase vascular contractile response to PE in the pedal artery. Since ergot toxicity in livestock mostly occurs after chronic exposure, and it is reported to result in abortion in pregnant animals, it is worthwhile to examine PE vascular contractile response during long-term and repeated exposure.

In the next experiment, we used pregnant sheep as our model for a 45-day exposure experiment. We evaluated the contractile response in different vascular beds: the pedal artery and the umbilical vasculature to assess our questions following a 45-day exposure to ergot alkaloids in pregnant sheep.

CHAPTER 4: Effect of 45-day Ergot Alkaloid Exposure on the Phenylephrine Contractile Response of Maternal Pedal Artery, Umbilical Artery and Umbilical Vein in Pregnant Sheep

This chapter contains the complete text of a manuscript that will be submitted for publication.

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4.1 Abstract

Ergot alkaloids have been known to cause devastating effects in livestock. Ergot alkaloids act on the biogenic amine receptors and result in vascular disturbance as well as decreased reproductive performance. The objective of this study was to evaluate the activation of α_1-adrenergic receptors in mediating vascular contractile response in the pedal artery (dorsal metatarsal III artery), the umbilical artery and the umbilical vein in pregnant sheep after long-term exposure to ergot alkaloids.

Twelve pregnant sheep were randomly placed into control and exposure groups (*n* = 6/group) after confirmation of approximately two months of gestation. The animals were maintained in a controlled environment with the ability to access to hay and water *ad libitum*. Daily ergot-contaminated feed pellets were given orally for 45 days to the exposure group (46.6 µg/kg BW total ergot alkaloids), while the control group received ergot-free pellets. The total concentration of major six ergot alkaloids (ergocornine, ergocristine, ergocryptine, ergometrine, ergosine and ergotamine) were determined using HPLC/MS at Prairie Diagnostic Services Inc., Saskatoon, SK, Canada. All animals were euthanized after 45 days of exposure. The maternal pedal artery from the left hindlimb, as well as an umbilical artery and vein from each animal, were carefully dissected and mounted in an isolated tissue bath. The vascular contractile response to a cumulative increasing dose of PE (α_1-adrenergic agonist) was compared between the two groups.

Chronic exposure to ergot alkaloids resulted in 70.6% and 91.3% increase in PE contractile response in the umbilical artery (Ctl EC_{50} = 3.962 x 10^{-6} M; Exp EC50 = 1.161 x 10^{-6} M, $P < 0.0001$) and the umbilical vein (Ctl EC_{50} = 7.889 x 10^{-6} M; Exp EC_{50} = 6.801 x 10^{-7} M, $P < 0.0001$), respectively, when compared to the control group. There was no significant difference in PE contractile response in the maternal pedal artery between the two groups ($P = 0.3927$). Fetal weight from the ergot exposed group was significantly lower than control group (Ctl 3.3 ± 0.17 kg; Exp 2.07 ± 0.13 kg, $P = 0.0002$). The increase in contractile response in the umbilical vein may result in decreased blood supply to the fetus causing decreased fetal weight. This is the first study to provide insight into the pathogenesis of the negative impact of ergot alkaloids on the umbilical vasculature in pregnant animals. Such an impact was seen at levels significantly lower than what is currently allowed by Canadian standards. Hence, it's possible that many ergot-induced livestock abortions are being overlooked by diagnosticians due to the assumption that the levels of ergot alkaloids in the feed are acceptable. It is, therefore, essential that these standards be revised to ensure the safety of the livestock industry.

Keywords: ergot toxicity, pregnant sheep, vasoconstriction, adrenergic receptors.

4.2 Introduction

Ergot poisoning in livestock is caused by prolonged consumption of ergot alkaloids within fungi-infected cereal crops and grains such as barley, wheat, and durum. In Western Canada, the majority of ergot alkaloids are produced by fungi in the genus *Claviceps* with the most commonly incriminated species being *Claviceps purpura* [64, 68]. Early signs of ergot poisoning in livestock include decreased milk production and lameness with the later progressing to complete hoof loss due to dry gangrene affecting the lower extremities[101, 102]. At this advanced stage, dry gangrene of the ear tips and tail end is often seen as these body parts are supplied by end arterioles [67, 69].

Although heterogeneous in their chemical structures, all ergot alkaloids share the "ergoline ring", the chemical structure of which resembles the physiological neurotransmitters: serotonin, dopamine, epinephrine and norepinephrine [29, 30, 37]. This resemblance allows ergot alkaloids to bind and activate the various receptors of these neurotransmitters resulting in an enhanced response with undesired clinical consequences. For example, ergopeptines, a group of ergot alkaloids, are known to be potent dopamine agonists activating its D_2 receptor. The repeated activation of this receptor is responsible for decreased milk production through the inhibition of prolactin secretion [76]. Similarly, the prolonged and repetitive activation of adrenergic and serotonergic receptors is thought to result in severe vasoconstriction ultimately causing gangrene in the extremities [34, 60, 98].

The consumption of ergot alkaloids is also known to have deleterious effects on pregnant animals [10, 80]. Clinically, signs of elevated rectal temperature, depression, weight loss due to reduced feed intake and progressive weakness are often seen and ultimately result in abortion [80, 101]. Reduced conception rate, shorter gestation period and reduced fetal weight have also been reported in farm animals [35].

Throughout pregnancy, the umbilical blood flow increases to meet the nutritional demands of the growing fetus. The umbilical vein supplies the fetus with nutrients from the dam while the umbilical arteries remove unwanted wastes. Thus, any impairment of blood flow through umbilical vasculature may have a negative impact on the fetus [19, 35, 38, 65]. The umbilical vasculature receives no sympathetic innervation [25]. Therefore, the regulation of vascular tone within the umbilical vasculature is mediated through several vasoactive catecholamines produced by the mother and the fetus. It is known that during fetal distress or fetal hypoxemia, the concentrations of these catecholamine change and alter umbilical flow [75, 78].

While several studies have shown vasoconstriction and reduced blood flow in umbilical veins after exposure to ergot alkaloids using ultrasound, few examined the mechanisms of these vasoconstrictive effects [13, 18, 22, 113]. The purpose of this study was to examine the effects of ergot alkaloid exposure during pregnancy on the maternal pedal artery, as well as the umbilical artery and vein of pregnant sheep. Another objective was to examine the role of α_1- adrenergic receptor in mediating these effects. We demonstrated that after a

45-day *in vivo* exposure to ergot alkaloids, the umbilical artery and vein, but not the pedal artery, display enhanced PE contractile response.

4.3 Material and Methods

4.3.1 Animals

All protocols involving the use of live animals have been reviewed and approved by the Animal Care and Ethics Committee at the University of Saskatchewan which complies with the guidelines of the Canadian Council on Animal Care.

Twelve healthy pregnant Katahdin dams each weighing approximately 60 kg were used to conduct this study and were randomly assigned into control and exposure groups (n = 6/group) after they were confirmed to be 2 months pregnant using ultrasound. Animals were first clinically examined with heart rate and body temperature recorded. A complete blood count was also performed on a jugular blood sample, which evaluated red and white blood cell counts as well as the platelet count and total plasma protein to ensure that the dams were healthy. Animals were kept indoors at an ambient temperature of 20 °C at the Animal Care Unit of the Western College of Veterinary Medicine and were housed together. They acclimatized for a 10-day period where they were daily provided with 100 grams of control pelleted diet in a bucket. At 7:00 am every morning, each animal in the treatment group was individually bucket-fed approximately 100 grams of a pelleted diet containing 28,000 ppb total ergot (46.6 µg/kg BW total ergot alkaloid

content) for 45 consecutive days while the control group received a normal control diet. Within the treatment diet, the concentrations of six ergot alkaloids; ergocornine, ergocristine, ergocryptine, ergometrine, ergosine, and ergotamine were determined at Prairies Diagnostic Services (Saskatoon, Saskatchewan, Canada) using HPLC/MS [56] with a detection limit of 1.25 ppb. See Table 4.4-1 for details. All animals were allowed access to water and alfalfa hay *ad libitum* for the duration of the experiment.

4.3.2 Tissue Collection

At the end of the exposure period, all dams were euthanized by captive bolt followed by exsanguination. Immediately after, full necropsies were performed, and a 10 cm-long segment of the umbilical cord was collected approximately 10 cm away from the fetal umbilicus from each animal. Another 10-cm segment from the pedal artery (dorsal metatarsal artery III) was carefully dissected and collected. All vascular segments were immediately placed into a container containing modified Krebs-Henseleit buffer buffer [in mM: 118 NaCl, 4.7 KCl, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 22.0 $NaHCO_3$, 5.0 glucose and 2.5$CaCl_2$; Sigma-Aldrich Canada Ltd. (Oakville, ON, Canada) (pH 7.4 gassed with 95%O_2, 5%CO_2 at 37˚C)] on ice. The Kreb's solution was later modified by adding 3 x 10^{-5} M desipramine and 1 x 10^{-6} M propranolol (Tocris Bioscience, Minneapolis, MN, USA) to deactivate catecholamine-neuronal uptake and β-adrenergic receptors, respectively [72]. All vascular segments were transported to the laboratory and placed into to a Petri plate containing modified Krebs-Henseleit buffer. All excessive connective tissue and the surrounding fat were carefully trimmed, and all vascular segments were

sectioned into approximately 2 to 3 mm rings. Each vascular ring was carefully placed into an individual triangular wire for suspension into an arterial tissue bath (Chengdu equipment manufacturing, China) containing 10 mL modified Krebs-Henseleit buffer (gassed with 95%O_2, 5%CO_2 at 37˚C) [72]. All arterial rings were maintained under a preload tension of 1 gram during equilibration with buffer replacement every 15 minutes. The viability of all vascular rings was examined by inducing contraction with phenylephrine at 5 x 10^{-4} M (Sigma-Aldrich Canada Ltd. Oakville, ON, Canada). Once steady state response was achieved, i.e., the tonic response to PE plateaued, tissues were washed every 15 minutes with normal modified Krebs' buffer until the original resting tension was restored.

4.3.3 Contractile Response

A cumulative PE contractile response was performed in each vascular ring by adding increasing concentrations of PE (1 x 10^{-8} M to 5 x 10^{-4} M) as previously described [72]. A PE treatment was added every 4 minutes allowing maximum response to be achieved for each particular concentration. Arterial rings were allowed to achieve maximum tension after each PE treatment followed by a 4-minute contractile plateau before the next treatment. After adding the last concentration, tissues were washed three times, and the resting tension was restored with buffer replacement every 15 minutes. All vascular segments were finally assessed for viability by exposure to PE at 5x10^{-4} M.

4.3.4 Data Collection, Analysis and Statistical Analysis

All measured isometric vascular contractions in response to PE were recorded as change in gram of tension on a Chart software (Chart V4.0.1) using a Powerlab data acquisition system (AD Instruments Inc., Colorado Springs, CO, USA). Tension responses were corrected for baseline and normalized as a percentage of the maximum contractile response induced by $5x10^{-4}$ M PE in order to adjust for differences in individual tissue size and architecture. Contractile response data were presented as percentage means ± *SEM* of the maximum contractile effect induced by 5×10^{-4} M. For each vessel type, a sigmoidal dose-response curve was plotted utilizing nonlinear regression with variable slope using Graph Pad Prism 6 (GraphPad Software Inc., La Jolla, CA). From each dose-response curve, the concentration required to produce 50% of maximum vascular contraction (EC_{50}) was determined. Statistical differences in EC_{50} among the different dose-response curves were calculated by the extra sum of square F-test where a *P*-value less than 0.05 was considered significant.

4.4 Results

The daily dose of total ergot alkaloids at 46.6 µg/kg BW was tolerated by all animals which remained healthy and showed no signs of anorexia, diarrhea, hyperventilation or lameness as previously reported in pregnant sheep exposed to ergot[35]. No gross or histological lesions were found in the lung, liver, kidneys, heart, spleen, intestines, fat, placenta, pedal or umbilical arteries in either of the treatment or control animals.

4.4.1 Maternal Pedal Artery Phenylephrine Dose Response Curve Compared between Ergot Exposure and Control Groups

No significant difference in EC_{50} was found between the control and the exposure groups in response to PE as shown in Figure 4.4-1. The PE contractile response was first observed at 1 x 10^{-7} M concentration in both groups and the maximum contractile response recorded at the highest PE concentration (5 x 10^{-4} M*)* was 14.81 grams in the control group, whereas the maximum contractile response recorded at the highest PE concentration in the exposure group was slightly higher at 18.2 grams. No significant difference was found in EC_{50} compared between the control and exposure group.

4.4.2 Umbilical Artery and Vein Phenylephrine Dose Response Curve Compared between Ergot Exposure and Control Groups

The mean contractile response of the umbilical artery to PE was first observed at 1 x 10^{-7} M in the control group and at 0.5 x 10^{-7} M in the exposure group. The maximum contractile response in the control group recorded at the highest PE concentration (5 x 10^{-4} M*)* was 1.30 grams, while the highest PE concentration in the exposure group yielded a maximum contraction of 4.5 grams. Ergot exposure resulted in a significant decrease in EC_{50} compared to control ($P \leq 0.001$) as shown in Figure 4.4-2.

The mean contractile response of the umbilical vein to PE in the control group was first observed at 1 x 10^{-7} M, whereas in the exposure group was observed at 0.5 x 10^{-7} M. The maximum contractile response recorded at the highest PE concentration (5 x 10^{-4} M) in

the exposure group was higher when compared to the control group which was recorded at 4.2 grams and 2.78 grams, respectively. Ergot exposure resulted in a significant decrease in EC_{50} compared to control ($P \leq 0.001$). Comparisons of PE contractile responses of the umbilical artery between the two groups are presented in Figure 4.4-3. Details of EC_{50} are for all groups are present in Table 4.4-2.

4.4.3 Phenylephrine Dose Response Curve Compared between Umbilical Artery and Vein in Ergot Exposure and Control Groups

Compared to the umbilical artery, the umbilical vein of sheep not exposed to ergot alkaloids displayed significantly higher PE EC_{50} (UA Ctl $EC_{50} = 3.96 \times 10^{-6}$ M; UV Ctl $EC_{50} = 7.9 \times 10^{-6}$ M, $P = 0.05$). See Figure 4.4-5. After ergot exposure the PE EC_{50} of umbilical vein was significantly lower than that of the artery. (UA Exp $EC_{50} = 1.16 \times 10^{-6}$ M; UV Exp $EC_{50} = 6.8 \times 10^{-7}$ M, $P = 0.002$). See Figure 4.4-6.

4.4.4 Result Tables

Table 4.4-1 The concentration of six ergot alkaloids determined in the ergot-contaminated feed pellets using HPLC/MS*. The total concentration of these alkaloids in feed pellet was used to formulate an oral dose (46.6 µg/kg BW) which was orally administered to each pregnant ewe once a day for 45 days.

Ergot Alkaloid	Concentration (ppb) Dry weight	Oral dose (µg/kg BW)
Ergocornine	3320	5.5
Ergocristine	12440	20.6
Ergocryptine	4730	7.8
Ergometrine	2110	3.5
Ergosine	1570	2.6
Ergotamine	4000	6.6
Total	28,200	46.6

*The detection limit for each alkaloid was 1.25 ppb.

HPLC/MS, high performance liquid chromatography and mass spectrometry; µg/kg BW, microgram per kilogram body weight; ppb, part per billion

Table 4.4-2 PE EC_{50} compared between ergot exposed and control sheep (n = 6/group) in dissected maternal pedal arteries, umbilical arteries and umbilical veins using an arterial tissue bath. Feed pellets were fed to both groups for 45 days with access to alfalfa hay and water *ad libitum*. Ergot exposed sheep received an oral dose of 46.6 µg/kg BW total ergot in ergot-contaminated feed pellets based on the levels of six ergot alkaloids determined previously. Control sheep received normal feed pellet.
For each treatment type, a sigmoidal dose-response curve was plotted using nonlinear regression which was used to calculate EC_{50}. Statistical differences in EC_{50} among the different treatment types were calculated by the extra sum-of-squares F-test were a *P*-value less than 0.05 was considered significant.

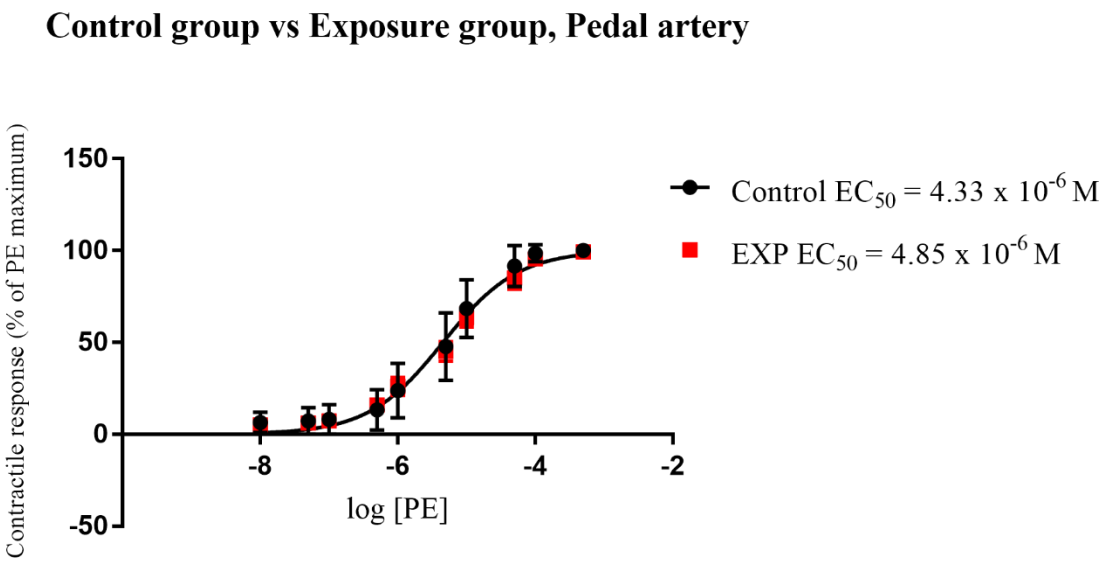
Blood vessels	**Control EC_{50} and 95% CI**	**Ergot Exposed EC_{50} and 95% CI**	***P*-value**
Pedal artery	4.33×10^{-6} (3.34×10^{-6} – 5.58×10^{-6})	4.85×10^{-6} (3.75×10^{-7} – 6.27×10^{-6})	$P = 0.39$
Umbilical artery	3.96×10^{-6} (2.88×10^{-6} - 5.4×10^{-6}) [a]	1.16×10^{-6} (9.69×10^{-6} - 1.45×10^{-5}) [a]	$P<0.0001$
Umbilical vein	7.9×10^{-6} (3.9×10^{-6} - 1.39×10^{-5}) [b]	6.8×10^{-7} (4.78×10^{-7}– 9.3×10^{-7}) [b]	$P<0.0001$

[a, b] Letters with the same superscripts are significantly different

EC_{50}, the concentration of phenylephrine producing 50% of the maximum contractile response; µg/kg BW, microgram per kilogram body weight; PE, phenylephrine; CI, confidence interval; nM, nanomolar

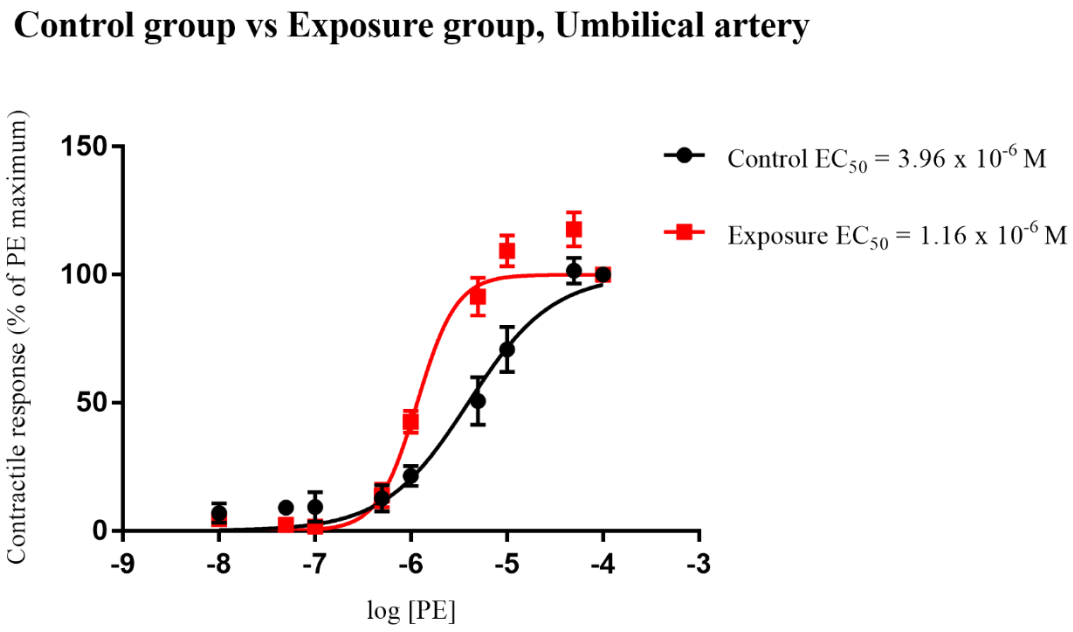
4.4.5 Result Figures

Figure 4.4-1 Mean contractile responses to increasing concentration of PE of the maternal pedal artery between control and ergot exposed group. The pedal artery was collected after a 45-day *in vivo* exposure to ergot-contaminated feed pellet (total ergot alkaloids 46.6 µg/kg BW) or normal feed pellet (n = 6 / group). There is no significant difference in comparison of the EC_{50} between the both group (P = 0.39). Contractile response data were presented as percentage means ± *SEM* of the maximum contractile effect induced by 5 x 10^{-4} M PE treatment.



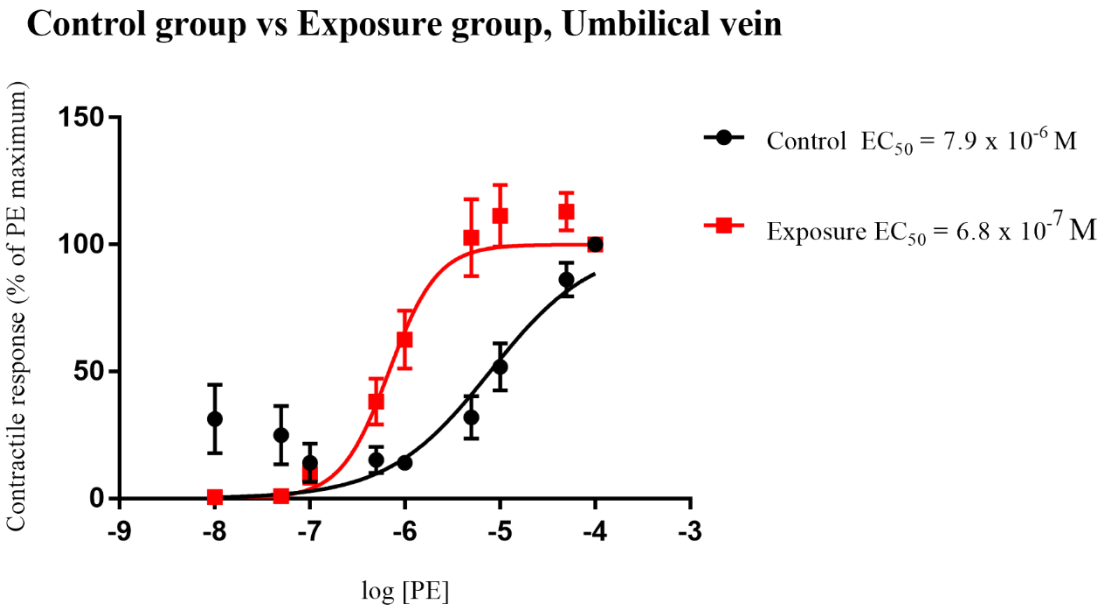
EC_{50}, the concentration of phenylephrine producing 50% of the maximum contractile response; PE, phenylephrine; µg/kg BW, microgram per kilogram body weight BW; CTL, control group; EXP, exposure group; M, Molar

Figure 4.4-2 Mean contractile responses to increasing concentration of PE of the umbilical artery between control and ergot exposed group. The umbilical artery was collected after a 45-day *in vivo* exposure to ergot-contaminated feed pellet (total ergot alkaloids 46.6 µg/kg BW) or normal feed pellet (n = 6 / group). Ergot exposure resulted in a significant decrease in EC_{50} compared to the control group ($P < 0.0001$). Contractile response data were presented as percentage means ± *SEM* of the maximum contractile effect induced by 5 x 10^{-4} M PE treatment.



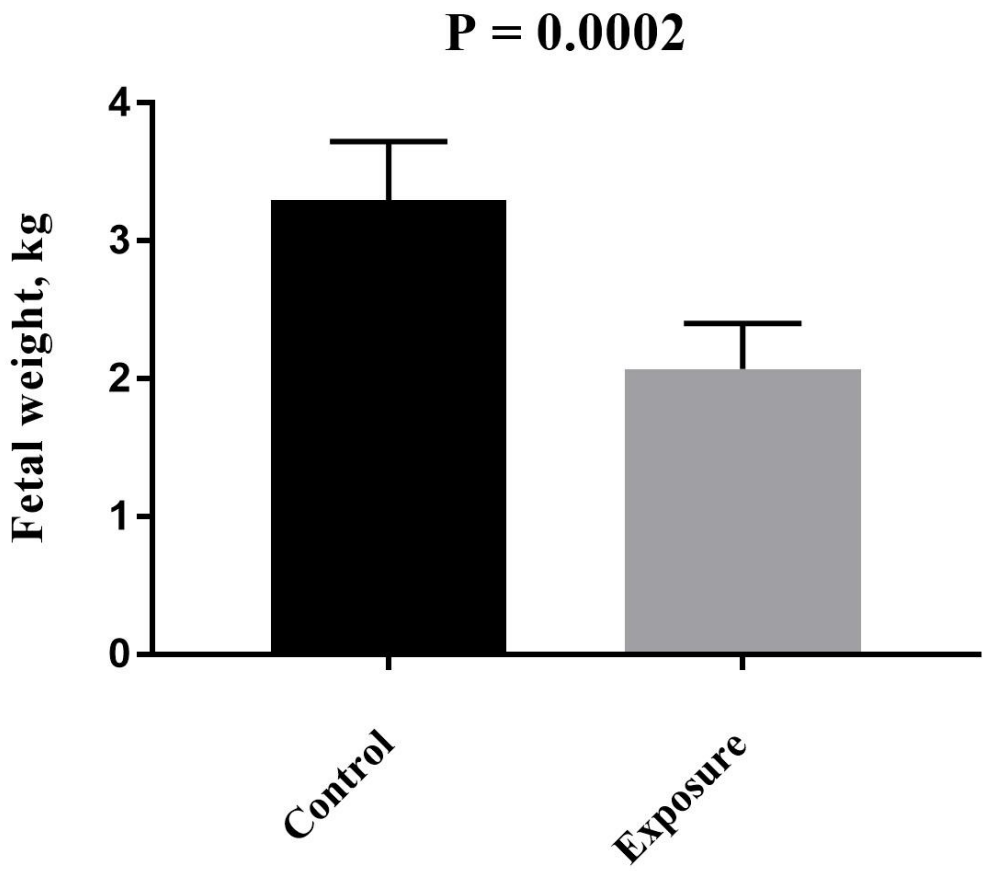
EC_{50}, the concentration of phenylephrine producing 50% of the maximum contractile response; PE, phenylephrine; µg/kg BW, microgram per kilogram body weight; CTL, control group; EXP, exposure group; M, Molar

Figure 4.4-3 Mean contractile responses to increasing concentration of PE of the umbilical vein between control and ergot exposed group. Umbilical vein was collected after a 45-day *in vivo* exposure to ergot-contaminated feed pellet (total ergot alkaloids 46.6 µg/kg BW) or normal feed pellet (n = 6 / group). Ergot exposure resulted in a significant decrease in EC_{50} compared to the control group ($P < 0.0001$). Contractile response data were presented as percentage means ± *SEM* of the maximum contractile effect induced by 5 x 10^{-4} M PE treatment.



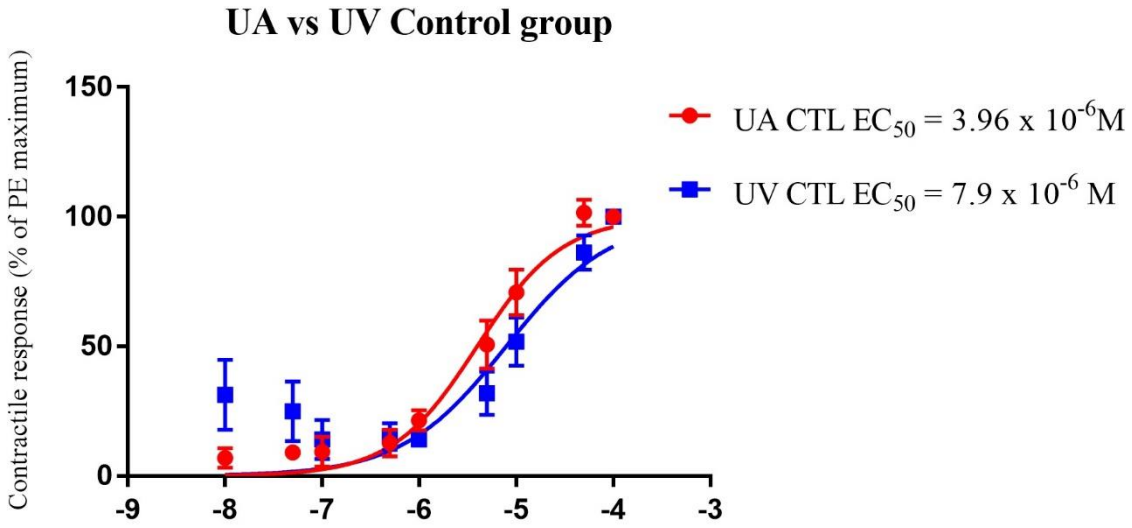
EC_{50}, the concentration of phenylephrine producing 50% of the maximum contractile response; PE, phenylephrine; µg/kg BW, microgram per kilogram body weight; CTL, control group; EXP, exposure group; M, Molar

Figure 4.4-4 Mean fetal weight (±*SEM*) from ewes fed ergot-contaminated feed pellets (total ergot alkaloids dose at 46.6 µg/kg BW) or normal feed pellet (*n* = 6/group) for 45-days during gestation. The mean fetal weight from the ergot exposed group was significantly lower than the control group (control, 3.3 ± 0.17 kg; exposure 2.07 ± 0.13 kg, *P* = 0.0002), (T-test, GraphPad Prism).



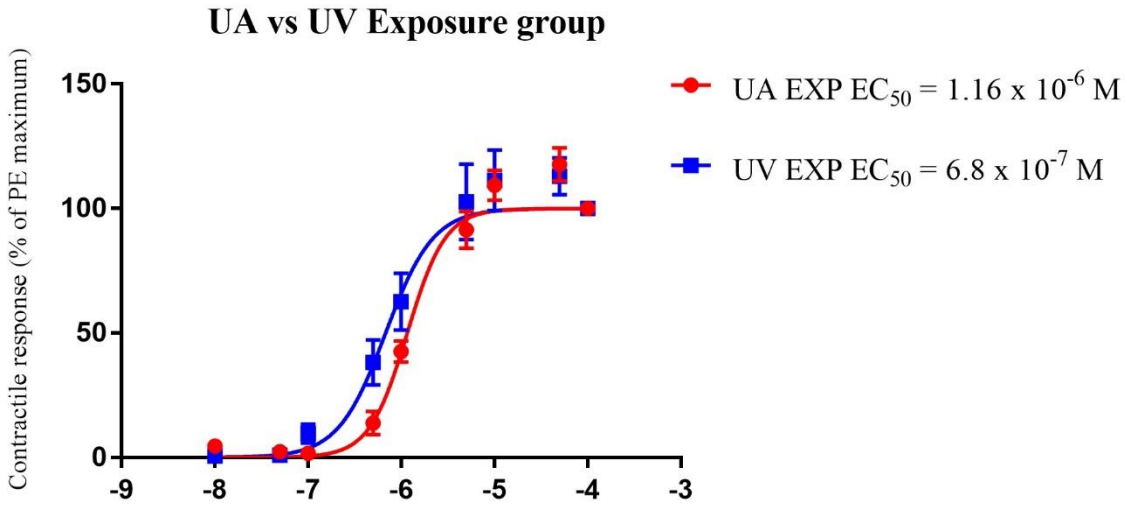
µg/kg BW, microgram per kilogram body weight; kg, kilogram

Figure 4.4-5 Comparison of mean contractile responses to increasing concentration of PE between umbilical artery and umbilical vein in control group. The umbilical artery and vein were collected after a 45-day *in vivo* study. Control group was fed a normal pelleted diet (n = 6). Umbilical artery PE EC_{50} was significantly lower than to that of umbilical vein (P = 0.05). Contractile response data were presented as percentage of means ± *SEM* of the maximum contractile effect induced by 5 x 10^{-4} M PE treatment.



EC_{50}, the concentration of phenylephrine producing 50% of the maximum contractile response; PE, phenylephrine; µg/kg BW, microgram per kilogram body weight BW; CTL, control group; EXP, exposure group; M, Molar; UA, umbilical artery; UV, umbilical vein

Figure 4.4-6 Comparison of mean contractile responses to increasing concentration of PE between umbilical artery and umbilical vein in exposure group. The umbilical artery and vein were collected after a 45-day in vivo study. Exposure group was given ergot-contaminated feed pellet (total ergot alkaloids 46.6 µg/kg BW) (*n* = 6). Umbilical vein PE EC_{50} was significantly lower than to that of umbilical artery (P = 0.002). Contractile response data were presented as percentage means ± *SEM* of the maximum contractile effect induced by 5 x 10^{-4} M PE treatment.



EC_{50}, the concentration of phenylephrine producing 50% of the maximum contractile response; PE, phenylephrine; µg/kg BW, microgram per kilogram body weight BW; CTL, control group; EXP, exposure group; M, Molar; UA, umbilical artery; UV, umbilical vein

4.5 Discussion

The PE contractile response in the umbilical vasculature following chronic exposure to ergot alkaloids using a sheep model was evaluated. A similar effect was also examined in the maternal pedal artery. PE is a selective α_1-adrenergic receptor agonist and, therefore, typically causes vasoconstriction in peripheral arteries [34, 85]. Human umbilical arteries are known to respond to adrenergic and serotonergic receptor stimulation resulting in vasoconstriction and altered blood flow [9]. Few studies have examined the response of sheep umbilical arteries to catecholamines. One study reported that serotonin and angiotensin were potent vasoconstrictors, while acetylcholine, epinephrine, and norepinephrine were less potent [21]. In addition, the epinephrine and norepinephrine contractile responses were blocked by adrenergic blockers [22]. Ergot alkaloids are known to cause poor reproductive performance in livestock resulting in fetal death, mummification and abortion [35]. Decreased fetal and organ weight have also been reported [18]. In mice, ergot alkaloids have been shown to cause failure of implantation and return to estrous as well as pregnancy interruption when administered in the diet [62].

Since ergot alkaloids are known to cause vasoconstriction in peripheral arteries, it is plausible to think that they would have similar effects on the umbilical vasculature during pregnancy. However, to the authors' knowledge, only a single report examined these effects in umbilical arteries. Dyer examined the contractile response to ergovaline in isolated bovine uterine and umbilical arteries and reported that the contraction developed slowly over 2 hours and did not relax over a 3 hours period despite a repeated buffer

change indicating strong affinity to the vascular receptor. Interestingly, this contraction was antagonized by ketanserin tartrate, a non-selective antagonist of 5-HT_2 receptors, but not prazosin or phentolamine, α-adrenergic antagonists. This study concluded that 5-HT_2 and not adrenergic receptors, were responsible for vasoconstriction [22, 113]. Contrary to the previous report, our study found that both the umbilical artery and vein displayed enhanced PE contractile response, i.e., more vasoconstriction at α_1-adrenergic receptor, compared to control blood vessels after chronic exposure. Our study suggests that α_1-adrenergic receptors play an important role in mediating the effects of ergot alkaloids. Receptors activation may, in part, explain the reproductive problems seen in livestock after chronic exposure.

It is important to note that the previous report studied the vascular effects of a single pure alkaloid, i.e., ergovaline, added to isolated arterial rings and not after actual oral exposure. In essence, it is known that compounds are modified by various physiologic processes after oral administration and a small change in the chemical structure of a compound can lead to altered potency or spectrum of activity [54]. Therefore, the previous report does not take into account the real *in vivo* vascular effects of ergot alkaloids after their metabolism or the combined effects of multiple ergot alkaloids, typically present in a regular diet. Very little is known about the metabolism of ergot alkaloids and whether the metabolites are vasoactive. However, there is evidence that ergot alkaloids bioaccumulate in tissues and the prolonged duration of their action could be related to the presence of active metabolites and tight tissue binding [48, 53, 83]. Others have also shown that alkaloid metabolism may result in decreased potency. For example, compared to

ergotamine, dihydroergotamine has been found to be less potent as a vasoconstrictor with α-adrenergic antagonistic activity [97]. Thus, it appears that the change in potency is likely dependant on the specific alkaloid and metabolite involved. Moreover, the most abundant ergot alkaloids in Western Canada do not include ergovaline, which is a dominant ergot alkaloid found in endophyte-infected tall fescue.

It is interesting to note that both the umbilical artery and vein were affected after 45-days of exposure. In human medicine, fetal status is commonly assessed by performing doppler ultrasound on umbilical arteries. An abnormal umbilical flow is considered a marker for uteroplacental insufficiency and is associated with fetal hypoxia and acidosis [8]. Similarly, reduced blood flow in the umbilical vein results in decreased delivery of nutrients to the fetus resulting in fetal death [18, 19, 75]. Our report, in agreement with others [18, 35], found decreased fetal weight after 45-days of ergot exposure which could be explained by the reduced blood flow in the umbilical arteries and veins. Surprisingly, the PE contractile response in the maternal pedal artery was not different from control arteries after 45-days of exposure to ergot alkaloids. Other studies in non-pregnant animals have shown increased PE contractile response in the lateral saphenous vein of sheep [74].

Pregnancy is known to be associated with remarkable, yet reversible, changes in maternal circulation resulting in altered maternal blood volume, cardiac output and peripheral vascular resistance [65]. In human beings, it is estimated that cardiac output increases by 40%, a change that is typically associated with a marked endothelium-dependent

peripheral vasodilatation. This vasodilatation typically leads to 35% decrease in peripheral vascular resistance [25, 99] .

In sheep, cardiac output, heart rate, and blood volume were shown to be significantly higher while systolic and diastolic pressure was shown to decrease during pregnancy compared to the post-partum period [65]. These changes in cardiovascular function parameters are associated with alterations in the contractile response within arterial vascular beds. For example, it has been shown that pregnancy results in 75% increase in mesenteric blood flow. To accommodate for this change, Kim et al., reported an increased acetylcholine stimulated relaxation of mesenteric arteries of pregnant guinea pigs compared to non-pregnant animals [46].

The finding of no significant difference in pedal artery contractile response after ergot exposure could be related to the decreased peripheral vascular resistance associated with pregnancy. Alternatively, it is possible that during pregnancy, the α_1-adrenergic receptor may not be an important mediator of the contractile response in this artery after 45-days of ergot exposure. The lack of a contractile response could also be related to the relatively low dosage selected in this study. It would be interesting to examine the serotonergic contractile response in this vascular bed during pregnancy to see if it is also affected.

No abortion occurred, and no gross or histological lesions were found in the lung, liver, kidneys, heart, spleen, intestines, fat or pedal arteries were detected after 45-days of oral ergot exposure in our study. Contrary to our finding, Greatorex reported that pregnant

sheep receiving ergot alkaloids aborted and displayed tongue necrosis, intestinal inflammation and hemorrhage [35]. The difference between the two studies is likely related to dose and regimen used as the previous study used a dose (1000 µg of ergotamine/kg BW) 20 times higher than the one used in our study (46.6 µg/kg). This high oral dose was also repeated three to four times daily. It is also possible that the vasculature of the tongue and intestine are more sensitive to ergot alkaloids during pregnancy in sheep.

As expected, the umbilical artery, having thicker tunica media, displayed lower PE EC50 compared to the unbiblical vein in control animals. Surprisingly, the effect of ergot exposure was more pronounced on the umbilical vein which displayed lower PE EC 50 than the umbilical artery. This may indicate that the umbilical vein is more sensitive to the effects of ergot than the umbilical artery.

It is important to note that the dosage used in this study is below the Canadian guideline limit for ergot alkaloids in sheep diet which is 3000 ppb [27, 28]. The experiment was also performed indoors with an ambient temperature of 20°C. The current Canadian limit may overlook the negative effects associated with the consumption of ergot alkaloids in freezing climatic condition when peripheral blood flow is already restricted [14]. Therefore, the absence of clinical manifestations in this study does not indicate that the used level of exposure is safe for this species in Western Canada.

In conclusion, we report that umbilical artery and vein, but not the pedal artery of pregnant sheep display increased contractile responses after 45-days of oral ergot

exposure which is mediated by the activation of α_1-adrenergic receptor. Future studies may focus on the role of serotonergic receptors in mediating these effects.

4.6 Acknowledgement

This study could not have been possible without the collaborative efforts of Dr. Desai for his expertise and guidance in tissue bath experiment as well as Dr. Leonardi for his dedicated work during the clinical trial.

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CHAPTER 5: Discussion and Conclusions

Ergot alkaloids are known to be vasoactive causing severe vasoconstriction in livestock after the chronic ingestion of infected grains. The effect is thought to be mediated through the activation of adrenergic and serotonergic receptors. This thesis aimed to address two important questions which were not previously examined in the literature. The first was whether these ergot alkaloids are vasoactive after a single high dose acute oral exposure. Typically, studies examining the effects of ergot alkaloids *in vivo* require the prolonged consumption of ergot alkaloids in feed for several months in order to mimic the real exposure scenario. Such studies are costly and time-consuming; therefore, most studies rely on examining the effects of ergot alkaloids by directly applying them to dissected vascular beds. While this approach offers valuable information, it does not mimic the real exposure scenario of oral consumption. If acute exposure causes similar vasoactive effects as chronic exposure, then an acute exposure protocol may prove to be useful to evaluate the chronic effects without the need for prolonged exposure.

We expected that the acute exposure would mimic the vasoactive effects of chronic exposure and would be mediated by the activation of adrenergic and serotonergic receptors. To our surprise, while a similar vasoconstrictive response was seen after acute exposure, only the adrenergic receptors were involved in mediating this effect! This may indicate that the acute exposure scenario may be useful in studying the adrenergic, but not the serotonergic effects of ergot alkaloids. One, however, has to be cautious not to completely dismiss the idea of serotonergic activation after acute exposure as the sample

size in this study was relatively small. Another interesting and yet unexpected finding of the acute study was that ergot exposed animals were more responsive to the effects of the α_1-adrenergic blocker (TE) compared to control animals, which may indicate this blocker may be useful in reducing the vasoconstrictive effects in ergot exposed animals. It may also indicate that ergot alkaloids may shift in their effect from being agonists, or even partial agonists, to becoming complete antagonists in the presence of TE! In support of this, it has been shown that ergotamine may act as a partial α_2-agonist and antagonist in dog femoral and saphenous veins, respectively [12]. The response to TE with vasodilation in affected species may also have diagnostic clinical implications. It would also be interesting to examine how TE affects other biological processes affected by ergot alkaloids such lactation and pregnancy.

One limitation of this study was our inability to accurately quantify the exact dose of exposure within ground sclerotia. Our analytical methods allow for the quantification of only six ergot alkaloids which are claimed to be the most abundant within sclerotia. While being abundant could be an indication of clinical significance, it is known that the vasoconstrictive potency of these alkaloids varies significantly with some being 100 times more potent than others. For example, ergovaline, which is a dominant alkaloid in infected tall fescue, has a similar potency to ergotamine which is commonly detected in ergot sclerotia. However, other ergot alkaloids such as ergocristine and ergocornine are ten times less potent than ergotamine. Ergocryptine is reported to be the least potent from all major six ergot alkaloids examined in sclerotia. In addition, the interaction of each ergot alkaloid to receptors is variable. For example, ergocristine can act on both α_2-

adrenergic receptors and α_1-adrenergic receptors. Also, ergotamine has an effect on both adrenergic and serotonergic receptors. Therefore, our calculated total dose of ergot alkaloids may not accurately reflect the vasoactive potency of the present mixture, which is reported to have more than 40 different ergot alkaloids in sclerotia[27, 28]. Nonetheless, our approach seems to be more clinically and scientifically relevant as it mimics the real exposure scenario and takes into account the pharmacokinetic effects of ergot alkaloids. Our approach also considers other interactions which may affect receptor binding such as the presence of other inhibitors or stimulators administered medicinally.

Another important aspect that this *in vivo* protocol provides is the effects of alkaloids considering the alkaloid metabolism taking place in the live animal. Some alkaloids may become more potent while others may be less potent after metabolism. For example, compared to ergotamine, dihydroergotamine has been found to be a less potent vasoconstrictor with α-adrenergic antagonistic activity [97].

Very little is known about the metabolism of ergot alkaloids and whether the metabolites are vasoactive. However, there is evidence that ergot alkaloids bioaccumulate in tissues and the prolonged duration of their action could be related to the presence of active metabolites and tight tissue binding [48, 53, 83]. Others have also shown that alkaloid metabolism may result in decreased potency [97]. Another limitation of this study was the sample size which was relatively small, still, significant detectable differences were found between groups.

The second important question in this thesis was whether vasoconstrictive effects are seen in the umbilical vasculature of pregnant animals after 45-days of oral ergot exposure. To our knowledge, no previous studies have addressed this important question despite the fact that ergot exposure is known to reduce fetal weight and to cause abortion. This study was limited to the effects on α_1-adrenergic receptors within the umbilical vasculature.

After a 45-day oral exposure in pregnant sheep, ergot alkaloids caused a significant increase in PE contractile response in both the umbilical artery and vein, but not in the maternal pedal artery. The increased contractility within the umbilical vein, associated with the finding of a decreased fetal weight, may indicate that ergot exposure may reduce blood supply resulting in decreased nutrients reaching the fetus and decreased fetal growth. The increased contractility within the umbilical artery may also have detrimental effects on fetal health as it may result in decreased waste removal from the fetus. The finding of no change in the maternal pedal artery contractility despite relatively long-term exposure to ergot alkaloids is likely related to the decrease in peripheral vascular resistance often associated with physiological changes during pregnancy. This is in contrast to what is seen in non-pregnant animals where pedal artery vasoconstriction after prolonged ergot exposure often leads to gangrene. It is also possible that the lack of effect reflects differences in receptor expression within the vasculature, changes between ergot and receptor interaction or altered metabolism and excretion during pregnancy. A less likely possibility is that the absence of effect is related to small sample size.

Although the current study contains a small sample size, the findings suggest that repeated exposure to ergot alkaloids, even at low concentrations, can be detrimental to livestock productivity. Fetal nutrient restriction is an important cause of increased neonatal morbidity and mortality in livestock [19]. Decreased fetal or neonatal weight also results in decreased average daily weight gain resulting in significant losses to producers [20]. Low birth weight has further been associated with increased pre-weaning mortality and increased incidence of infections. As such, addressing the issue of high ergot levels in feed would likely have a significant economic impact for livestock producers, increasing livestock productivity, decreasing treatment cost and increasing profitability[19, 20].

It is important to note that sheep are known to be less susceptible to ergotism than cattle and horses [27]. It is thus likely that the vascular effects described in both studies performed here will be amplified and probably more clinically noticeable in these other two species [45, 72]. This indicates that gangrene and abortion in these other species may occur at even lower concentrations than the ones used in this study emphasizing the need to create species-specific standards to address the differences in species susceptibility. The fact that the effects seen in the second study occurred at concentration levels lower than what is permitted in livestock feed indicates the need to revise these standards to reflect the safety concerns associated with low-level exposure.

Notwithstanding these limitations, the study suggests that allowable limits of ergot alkaloids in livestock feed need to be revised with consideration of the climate, species

and physiological stage of animals. The findings of these studies also shed light on the clinical implication of using adrenergic blockers as a treatment for ergot toxicity. Future studies should focus on examining the role of serotonergic receptors and other adrenergic receptors in mediating the effects of ergot alkaloids on the umbilical vasculature. It would also be interesting to examine the length of time the vasoconstrictive effect last within all examined vascular beds.

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